

THE NEW PHYTOLOGIST

A BRITISH BOTANICAL JOURNAL

EDITED BY

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CAMBRIDGE

IN CONSULTATION WITH THE STAFF OF
THE CAMBRIDGE BOTANY SCHOOL

VOLUME XXV

PAGES viii AND 380

WITH 8 PLATES AND NUMEROUS FIGURES
IN THE TEXT

LONDON

WHELDON & WESLEY, LTD.

2, 3 & 4, ARTHUR ST., NEW OXFORD ST., W.C.2

1926

PRINTED IN GREAT BRITAIN

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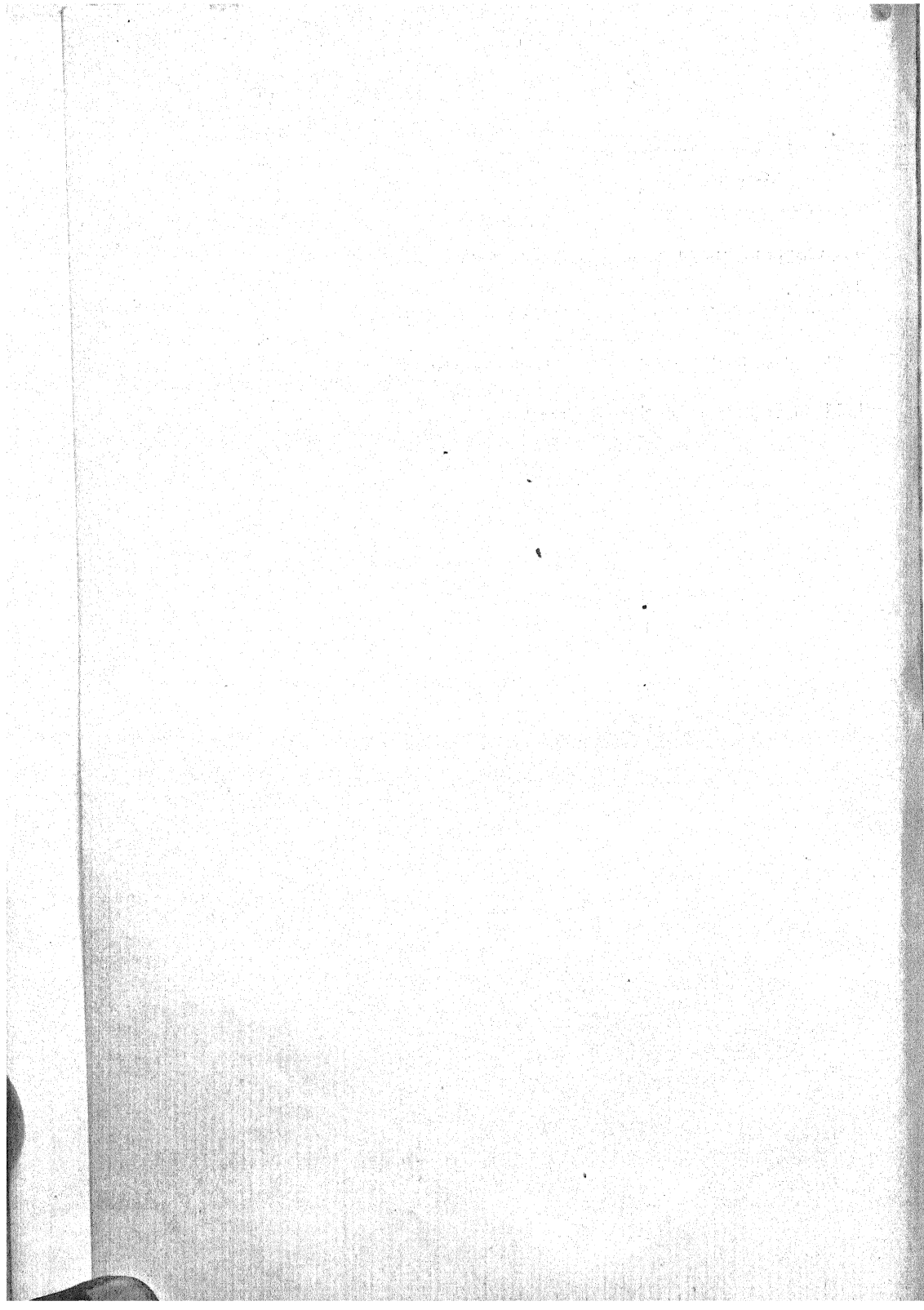
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THE NEW PHYTOLOGIST

VOL. XXV, No. 1

17 MARCH, 1926

MYCORRHIZA

By M. C. RAYNER

CHAPTER I

Introductory—Historical—The Early Period: 1840–1880—Schleiden—Reissek—The *Monotropa* controversy—De Bary's theory of symbiosis.

STRICTLY considered, knowledge of mycorrhiza dates from the year 1885, when Frank first applied the name to roots of trees showing a regular and characteristic infection by fungal mycelium.

Taking a broader view, the history of the subject falls naturally into three periods, the order of which coincides roughly with progressive changes in the points of view actuating research on the subject. Firstly, there is the period previous to 1880 during which a number of observations bearing on the subject were recorded, the full significance of the facts being either unappreciated at the time or becoming the subject of considerable controversy among botanists. Most of these early observations fall within the period 1840–1880.

A second period began in 1881 with the work of Kamienski on *Monotropa hypopitys*, and the researches, experiments and speculations of Frank and his fellow workers, and extended over the last two decades of the nineteenth century. This was essentially one of observation and speculation, largely dominated by the work of Frank and his school, and it passed without a break into what may be called the Modern Period, marked especially by the application of modern experimental methods of research to the problems presented by mycorrhiza plants.

THE EARLY PERIOD: 1840–1880.

Records of observations made during the first decade of this period and in that preceding it are of interest historically. Some of the earliest noted the presence and described the structure of curious thread-like bodies within the root cells of plants. In certain cases,

subsequent observers identified the threads as fungal filaments and noted their regular occurrence in the roots of vascular plants; in others the real nature of the "hairy structures" associated with roots remained doubtful and aroused considerable controversy among contemporary botanists.

Among the earliest observations bearing on the subject are those of Meyen (1829), Nägeli (1842) and Schleiden (1842). The two first-named workers put on record the regular appearance of fungi within the cells of a number of plants but did not arrive at correct conclusions as to their significance or mode of origin within the cell.

Meyen's attention was attracted to the root tubercles of Alder which he believed to be parasites of similar habit to members of the Balanophoraceae and Orobanchaceae, although lower in development and systematic position. Nägeli recorded the presence of filamentous fungi in the roots of various species of *Iris*, and also in other genera,—“In diesen parenchymatischen Zellen lebten 3 verschiedenen Arten von Pilzen.” He described intracellular flask-shaped bodies produced at the ends of the threads, and placed these root fungi in a new genus, *Schinzia*.

The Orchids were among the first plants to attract attention in respect to the anomalous structure of their root cells. Link (1840) figured cells containing fungus mycelium from the young seedling of *Goodyera procera*, but was quite ignorant of the real nature of the granular cell inclusions which he observed.

The observations of Schleiden (1842) on the roots of *Neottia nidus avis* are of particular interest as being the first bearing specifically on the subject of root infection by fungi. He described with astonishing accuracy the microscopic appearance of the various types of cell in the cortical tissues of the roots of this Orchid, their slimy contents and the presence of thread-like structures in many of the cells. Describing the structure of the threads more fully he noted that they were hollow and branched freely, and also that they sometimes formed a tangled skein the branches from which ended blindly within the cells. Lacking accurate information as to development, Schleiden expressed no opinion as to the real nature of these threads, beyond suggesting a possible analogy with the cells showing spiral markings described by Gottsche (1843) in the tissues of a Liverwort, *Preissia commutata*. With admirable frankness he thus put on record his sense of the remarkable nature of the root tissues and his ignorance of their significance to the plant:—“Ueber die Bedeutung dieser eigenthümlichen Bildungen weiss ich gar nichts zu sagen.”

It was Reissek (1846) who first attempted to determine the origin and real nature of the thread-like structures present in the root cells of certain vascular plants; incidentally he attempted to correlate the observations of Meyen and Nägeli just mentioned with the results of his own more extended researches. Noting their regular appearance in the roots of many Monocotyledons and Dicotyledons, Reissek reached the correct conclusion that the thread-like structures were undoubtedly of fungal nature. He concluded, further, that the root fungi (Wurzelpilzen) reached their highest development in the underground roots of Orchids, and, although relatively common in the roots of other Monocotyledons and in Dicotyledons, were present only in a rudimentary condition in plants other than the Orchids:—"Hier sind nur die Keime desselben vorhanden."

It was mainly upon researches on Orchid roots that Reissek based his remarkable conclusions as to the origin of these intracellular fungus threads. He described their development from granules included among the ordinary cell constituents and in his view identical with the primordia of starch grains and chlorophyll grains. The granules were held to be fungus spores that developed into spindle and rod-like structures and finally to a skein of hollow threads as in the root cells of *Neottia*. Stages in this process were traced and figured in the root cells of *Goodyera discolor*. Presumably the spores originated from the granules *de novo*, since it is elsewhere explicitly stated by the author that the mycelium did not form spores within the cells but did so only when grown in the air.

It is somewhat surprising to find that Reissek attempted to extract and cultivate the intracellular mycelium present in roots. In view of the technique adopted, not so surprising to learn that among the fungi isolated were a species of *Fusiformium* (from *Orchis morio*) and species of "*Botrytis*, *Penicillium* and *Cladosporium*" from other Orchids. Spores of various species of these fungus genera are widely distributed both in air and in the soil; species of *Penicillium* and *Cladosporium* in particular are among the commonest constituents of the epiphytic mycelial flora of roots and appear regularly when attempts are made to isolate the true root endophytes by unsuitable methods. One or other member of the group has been recorded as a specific endophyte by many investigators subsequent to Reissek, but no satisfactory evidence has ever been provided that any species of the genera named is concerned in the formation of mycorrhiza.

This contribution by Reissek is notable as the first full and at all

accurate account of the association between root cells and fungus mycelium now known as mycorrhiza.

Study of these older papers recalls the views current at the time respecting the origin of Fungi and Bacteria. By the early botanists they had been regarded not as living organisms but as *lusus naturae*. Subsequent to their tardy recognition as true members of the vegetable kingdom, they were for long believed to be produced by spontaneous generation from inorganic material, or to arise from organic substance not in itself of fungoid or bacterial nature. This theory assumed that constituent particles of living cells belonging to the higher organisms could continue to live after the death of the body of which they had formed part, and could develop under favourable conditions into fungi and bacteria that were capable of producing germs and of giving rise to progeny specifically resembling the parents (Ehrenberg 1820).

The microzyme theory of Bécamp, a logical statement of this point of view, assumed the existence of very minute bodies—"granulations moléculaires" or "microzymes"—in the substance of plants and animals, occurring everywhere, enjoying an almost unlimited duration of vitality, and giving rise under suitable conditions to bacteria, sprouting fungi, and similar forms. The thesis embodying these views in detail was first published in Paris in 1864, reproduced in the *Transactions of the Medical Congress* at London in 1881, and republished in an extended form in Paris as late as 1883.

In England the attention of botanists was first attracted to the association of fungus mycelium with roots by a controversy respecting the alleged parasitic habit of the Yellow Bird's Nest, *Monotropa hypopitys*. In 1844 an English periodical—*The Phytologist*—published a number of contributions to a discussion on this matter, in the course of which attention became focussed upon the nature of the fibrous investment noticed upon the roots of *Monotropa*.

Luxford (1844) held that this plant obtained at least a part of its nourishment from "a layer of vegetable matter, consisting chiefly of the slowly decaying leaves of the beech, which are generally covered with a white byssoid fungus." Alone among the contributors to the discussion, Lees (1844) took the view that the plant was a true parasite. He figured a young plant of *Monotropa* growing from "its radical parasitical knob," and described in detail the hairy knobs upon the beech roots, "of all sizes from that of a pea, from which the little embryo *Monotropa* was sprouting, to that of a crab,

nourishing a full company of several plants." Lees described also the hairy fibres by which the *Monotropa* plant attached itself to the root of Beech. These fibres he regarded as special organs functioning as suckers, and he noted that the ball or "nidus" at the base of the *Monotropa* plant is obscured with "a hirsuture that appears like a byssoid fungus." (Fig. 1.)

Newman (1844) regarded the fibres as essentially part of the root itself and Wilson (1844) recorded his view that they represented the matted extremities of grasses with which the *Monotropa* roots were in contact. Alluding to his examination of the roots of plants collected near Southport (a locality where *Monotropa* is still found) and believed to be parasitic upon the roots of *Salix argentea*, he wrote as follows:—

...the mass composing the root of the Southport *Monotropa* had a white covering of a matted and somewhat woolly substance, supposed to proceed from the radicles of the grasses which grew with it.... There was not even contact, much less parasitical connection, between this white coating and the roots of the *Salix* contained in the sod.

In view of these very conflicting opinions, Rylands (1844) re-investigated *Monotropa* material from various localities and eventually reached correct conclusions regarding both the habit of the plant and the true nature of the fibrous investment of the roots. "The plant," he states, "is not parasitic; it has no organic connection with the 'nidi' of roots among which its own are developed." From a careful examination of the fibres he concluded also that the "byssoid substance is really fungoid, and performs no essential function in the economy of the *Monotropa*."

In order to test the correctness of his observations on *Monotropa*, he examined the roots of a number of other plants and reported as follows:—"the *really* fungoid matter found on the roots of groundsel, *Epilobium*, *Plantago*, etc., had so much resemblance to the substance in question, that it would be difficult by words to render the difference appreciable."

Rylands published careful drawings of the root of *Monotropa* with its fungus sheath and figured details of the hyphae. One of these drawings (Fig. 2) is of rather special interest in relation to modern work and will be mentioned again. With the help of Berkeley—the founder of British mycology—he referred three out of the four fungi distinguished upon the roots to known genera: the other was placed provisionally in a new genus, *Epiphagos*.

Ryland's observations seem to have temporarily exhausted interest in the subject in England but the controversy respecting the parasitic habit of *Monotropa* was renewed subsequently by various continental botanists. Thus, in a work on comparative anatomy by Chatin (1856) *Monotropa* was described as a parasite in the younger stages of growth; later it became detached from its host and grew saprophytically. The accurate observations of Solms Laubach (1868) demonstrated that no organic continuity could be detected between the roots of *Monotropa* and those of the tree beneath which it grew, however close the contact might be, and, therefore, that the former plant was not a parasite. Drude (1873) again described and figured a parasitic connection between the roots of *Monotropa* and those of *Abies excelsa*, an error of observation accounted for later by Kamienski. In the same paper Drude mentioned the mycelium associated with the roots of *Monotropa*, and compared it with the appearances described long previously by Schleiden in *Neottia*.

With the exception of these controversial records concerning *Monotropa*, the possible value of observations bearing upon the association of fungi with the roots of vascular plants seems to have escaped the attention of botanists for a number of years. Isolated observations by various continental botanists continued to place on record the presence of fungal hyphae in and upon roots, although the time was not yet ripe for an appreciation of the full significance of the observed phenomena. For example, from the year 1840 onwards, Theodor Hartig (1840-1851) had noted and put on record the webs of hyphae surrounding the tips of the fine absorbing roots in Conifers, and had observed also the network of mycelium between the cells of the outer cortex of the roots. He was mistaken, however, in his interpretation of the real nature of these structures, which he regarded as purely parasitic. Fabre (1855-1856) also had observed filamentous threads and skeins in the root-cells of various Orchids without realising their true nature, which indeed was first clearly recognised by Schacht in 1854.

Describing the roots of *Neottia*, Prillieux (1856) says:

Les cellules de la racine de *Neottia nidus avis* portent des nuclei d'une grosseur extraordinaire, et sur lesquels j'ai plusieurs fois distingué deux nucleoles;... Les cellules contiennent de la matière brunâtre renferment également des filaments enroulés sans ordre autour de la masse solide qui occupe le milieu de la cellule. Ces filaments sont creux; ce sont de véritables tubes dont on distingue avec certitude les parois.

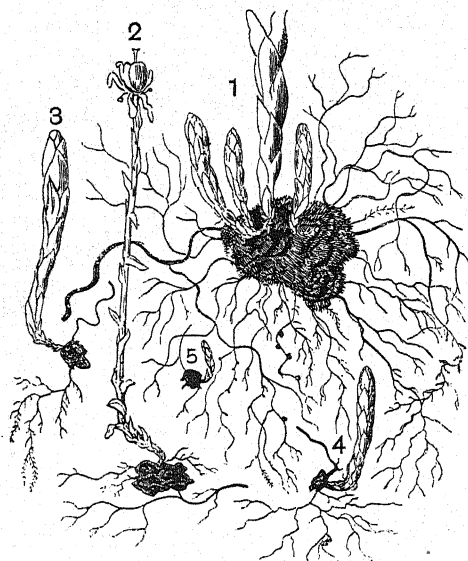


Fig. 1. Illustrations of the mode of growth of *Monotropa hypopitys*. (1) Base of a mature plant, 14 inches high, and three young unexpanded plants, growing from their radical parasitical knob. (2) Smaller plant in seed. (3), (4) and (5) Young plants growing from radical knobs. (Figures and description from Lées, *The Phytologist*, 1842.)

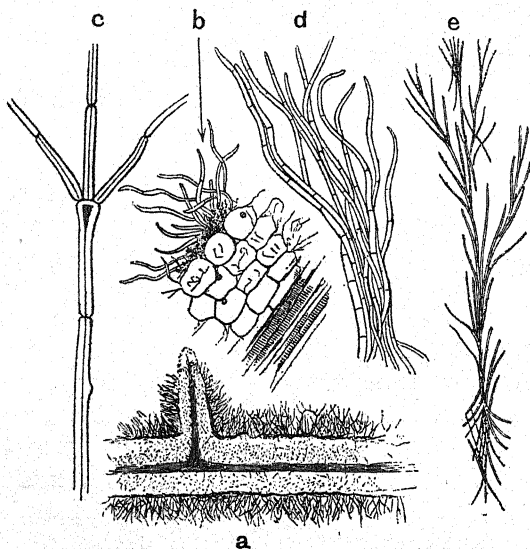


Fig. 2. (a) A section of the root of *Monotropa*, showing the internal continuation of the vascular tissue of the spongiote or fibril. (b) A portion of the same highly magnified, to show the connection of the flocci. (c) *Epiphagos luxfordii*, highly magnified. (d) and (e) The same, showing its adnate and fasciculate habit. (Figure and description from Rylands, *The Phytologist*, 1842.)

From the year 1860 onwards there was a great quickening of interest in aspects of biology relating to nutrition, more especially in those concerned with differences in the nutrition of plants as compared with animals. Before that date, the nutritive processes of plants and animals were believed to differ essentially,—the plant building up complex bodies from simple substances, the animal breaking these down again. The researches of Schultz (1861–1863) established the fundamental similarity of plant and animal protoplasm, thus leading to a search for the real causes for the differences believed to exist in the two groups in respect to nutrition. Following quickly upon this came a realisation of the specific property of green plants in relation to the manufacture from raw materials of substances suitable to be used as food materials by animals, with which was associated a newly awakened interest in exceptional modes of life and nutrition, e.g. in the behaviour of insectivorous plants, and in the presence of fungi and bacteria in the animal body.

Owing to their lack of chlorophyll and inability to utilise carbon dioxide, the Fungi stood out as possessing a mode of life fundamentally different from that of green plants. Observations by Pasteur in 1860 and 1862, by Nägeli in 1879 and 1883, and by Reinke in 1883 all showed that the former could use a great variety of carbon compounds as food materials. In 1874 so noted a botanist as Hooker, speaking of the Fungi, observed:—"these plants seem to invert the order of Nature and to draw their nutriment, in part at least, from the animal kingdom, which it is held to be the function of the vegetable kingdom to sustain" (Hooker, 1874).

The increased interest in problems of nutrition and the rapid advance of knowledge in this branch of biology were responsible also for the attention attracted to symbiotic phenomena generally from 1860 onwards. The true nature of the Lichen thallus, foreshadowed by de Bary in 1866, had been confirmed by Schwendener (1868, 1870) and established by the experimental researches of Stahl (1879), Reess (1879) and others. In 1879 the word *symbiosis* was coined by de Bary to cover all cases involving the living together of dissimilar organisms however loose the association. In his own words:—"...eine Betrachtung der Erscheinungen des Zusammenlebens ungleichnamiger Organismen, der Symbiose, wie man kurz und allgemein sagen kann..." Thus defined, the term includes all cases of parasitism, and was clearly so intended and used by de Bary himself:—"Die bekannteste und exquisiteste Erscheinung der Symbiose ist der vollständige Parasitismus" (de Bary, 1879). The

above extracts are taken from an address delivered by de Bary to a meeting of a German natural history society at Cassel in 1879. It was published as a separate not always easily accessible for reference, and preceded by a few years only the general recognition of mycorrhiza as a typical example of the phenomena included by de Bary under the term symbiosis.

CHAPTER II

The Second Period: 1880-1900—Kamienski—Frank: recognition of mycorrhiza as a morphological entity—Classification into ectotrophic and endotrophic forms—Theory of beneficial symbiosis—The relation of Truffles and other fungi to Mycorrhiza—Hartig's theory of parasitism.

THE SECOND PERIOD: 1880-1900 circa.

The gradual recognition of root infection as a regular and extremely widespread phenomenon among vascular plants began during the first years of this period. Simultaneously, interest was awakened in its physiological significance and experimental research on the subject was undertaken in more than one of the continental schools of botany.

Many different circumstances contributed to this result. There was already in existence a considerable body of sporadic observations recording the presence of mycelium in and upon roots. Of these a large proportion had been made upon species which showed marked peculiarities of structure and habit, and to which the attention of botanists had long been directed in respect to their anomalous mode of nutrition. For example, the non-chlorophyllous orchids *Neottia nidus avis*, *Corallorrhiza* and *Epipogon* had already been worked at by Schleiden (1842), Schacht (1852, 1854), Irmisch (1853), Prillieux (1856), Drude (1873) and Reinke (1873). The controversy among English botanists concerning *Monotropa* has been mentioned (p. 4) and will be more fully discussed below.

Contemporary observers could hardly fail to relate the invariable presence of mycelium in these remarkable plants with the anomalous mode of nutrition involved by their lack of chlorophyll, especially at a time when the attention of botanists was focussed upon problems of plant nutrition. The development of abnormal roots by certain trees and the presence of mycelium believed to be that of a parasitic fungus upon such roots had also attracted the attention of more than one observer.

In another field of work the discovery of the dual character of the Lichen thallus and the attention directed to symbiotic pheno-

mena by de Bary had stimulated interest in relationships between green and non-green plants. The root tubercles of Leguminosae and those of *Alnus*, *Eleagnus* and other trees challenged attention, as did also the causes of gall-formation in general. Furthermore, the work of Pasteur and his school had prepared the way for a study of pathogenic conditions in vascular plants and their causation by parasitic fungi. The cytological changes in plant cells subjected to invasion had also come under observation and were available for comparison with those in mycorrhiza tissues, while the improvement in technique and optical apparatus was reflected in the increased accuracy of microscope observations and also in their greater cytological significance. Evidently the time was ripe for a more general recognition of the prevalence of fungal infection in roots, the possible significance of this phenomenon in plant nutrition, and its relation to parasitic infection of the ordinary kind.

Doubtless many of these circumstances were operative in leading Kamienski to re-investigate *Monotropa hypopitys*, a species which, in spite of the attention lavished upon it by botanists, was still the subject of many conflicting statements in botanical literature. The extent and scope of this earlier work on *Monotropa* may be judged from the account already given and from the following brief review.

The anatomy had been investigated by Unger (1840), who recorded the absence of true vessels from the wood, an observation not confirmed either by Solms Laubach (1862) or by Drude (1873). A paper describing the presence of "einen eigenthümlichen Stoff" in *Monotropa hypopitys* was contributed by Reinsch (1852). The minute seeds early attracted attention; their structure was described by Muller (1848), who, however, mistook the combined endosperm and embryo of the seed tissues for the embryo alone. Solms Laubach (1874) and Koch (1882) recognised the distinction between embryo and endosperm, the structural details supplied by the latter being amply confirmed by Kamienski in his memoir published in the same year. The supposed parasitic habit of the plant had long been a subject of controversy. Some of the views current have been mentioned; those of other observers may be summarised as follows:— In 1840 Unger had placed *Monotropa* in his seventh order of parasites, a view confirmed by Brandt (1869), who included this species in the same class as the Orobanches. Chatin (1865) had described and figured the seed, and had published an account of seedling development. According to this observer the plant was a parasite in the young stages, penetrating the root of the host by

means of its thread-like base: at a later stage of growth, the basal part of the seedling perished, the organic connection between parasite and host disappeared and the mature *Monotropa* plant was nourished entirely from the soil. Drude had reached a somewhat different conclusion. Having observed young seedlings of *Monotropa hypopitys* growing among Pine needles, he concluded that the plant was a saprophyte in the early stages, becoming parasitic in the mature condition. He described and figured *Monotropa glabra* as a parasite, the roots of which penetrated those of Beech and Pine and drew nourishment therefrom. As in the earlier controversy on the same subject among English botanists the last word lay with those who held a contrary view respecting the habit of the plant. Thus, in discussing *Monotropa uniflora*, W. Hooker threw doubt upon its parasitism, pointing out that seedlings could be raised from seed sown upon humus, independently of any host¹.

Following upon a special study of the vegetative organs Schacht (1854) was even more explicit, stating that *Monotropa* formed no organic connections whatever with a host and was not parasitic at any stage of its life; he concluded that, like *Neottia*, it can nourish itself on the decaying products of certain plants and for that reason is always found in their near neighbourhood. Solms Laubach (1862) could find no haustoria or other evidence for regarding the plant as a parasite, a view in which Duchartre (1882) concurred. From this brief survey, it is evident that the structure and habit of *Monotropa* still offered a promising field for investigation, especially in respect to the fungus investment of the roots. Advantage of this fact was taken by Kamienski, whose classical researches on the subject, although they ante-dated the bestowal of the name "mycorrhiza" upon a corresponding structure in the roots of Cupuliferae by Frank in 1885, must be regarded as the first milestone on the route leading to the present knowledge of the subject.

Kamienski published a preliminary paper in 1881, in which, after criticising some of the views expressed by earlier workers, he gave a detailed anatomical description of the vegetative organs of the plant and described the mycelial sheath present on all the roots. The conflicting nature of the earlier views were thus described:—
"...les résultats donnés par les auteurs de ces différents travaux sont si peu concordants, qu'il est absolument impossible d'en conclure quelque chose de positif au sujet de la structure, du dé-

¹ Seedlings of *Monotropa uniflora* were raised at the Glasgow Botanic Garden on soil brought from Montreal (see Hooker, 1825).

veloppement ou de la manière de vivre de cette plante....” His own observations confirmed the view that haustoria were not present, and led him to conclude that in respect to nutrition the plant was a non-chlorophyllous saprophyte living upon humus in the soil. The structures described and figured by Drude as haustorial connections in *Monotropa glabra* he interpreted as fungus-deformed roots of Conifers growing among those of the former plant and closely resembling them in respect to the presence of a fungal sheath. In his account of the mycelial investment of the root of *Monotropa hypopitys* he noted that it was present in all roots, forming in each a continuous sheath which thinned out over the extreme tip:—
“Toutes les parties les plus actives de la racine sont recouvertes d’une couche épaisse et dense d’un mycélien qui ne permet pas aux racines d’avoir un contact direct avec le sol.” With reference to the absorption of food material, Kamienski recognised that all soluble nutrients taken in by the roots from the soil must pass through this fungal zone, and he raised the question of its beneficial or other effect upon the plant. A satisfactory answer to this question, he admitted, could be supplied only by the extension of his researches to include the raising of plants free from fungal infection by means of seed cultures.

This preliminary paper was followed by a fuller account including a critical survey of earlier work and a full and accurate description of the morphology and anatomy of the vegetative organs illustrated by excellent drawings (Kamienski 1882). As regards the habit, Kamienski confirmed his earlier view that no evidence whatever existed that the plant was a parasite. This memoir may therefore be regarded as closing the long controversy upon this matter, although it left unexplained the exact nature of the *saprophytic* mode of nutrition assumed to exist, and contributed no certain information upon the part—if any—played by the root fungus in the nutrition of the vascular plant.

Experimental work in seed germination undertaken by Kamienski gave no results. Seeds sown on many different substrata, e.g. humus, peaty soil, and manure, all failed to germinate, and the conclusions expressed in this paper depend, therefore, upon facts of observation only. Historically, they are of interest, inasmuch as the relation between root fungus and vascular plant was, for the first time, clearly defined as differing essentially from that existing in cases of ordinary parasitism, and there can be no doubt that the rights of priority in this matter belong to Kamienski rather than to Frank.

In relation to the subject of root infection generally, the paper has somewhat special interest because the author noted and carefully described the fine, fungus-infected roots of the trees under which *Monotropa* commonly grows, especially those of Beech, of which he supplied a figure. He commented upon the arrest of growth and increased branching exhibited by these roots, and also upon the absence from each of a typical root-cap and the invariable presence of a continuous sheath of interwoven hyphae, branches from which penetrated between the cells of the epidermis and formed a network separating the cortical cells one from another. In Conifers generally, and especially in Pine, the multiplication of lateral branches was very conspicuous and was noted as exactly resembling the typical dichotomy found in Lycopodiaceae.

More especially was Kamienski impressed by the similarity of habit, texture, and structure in the roots of Beech and those of *Monotropa* with which they were often closely associated. He believed, doubtless correctly, that to this fact was due Drude's error of observation respecting the existence of haustoria in the latter.

Similar roots showing abnormal structure had already been noted and described for several different trees by Janczewski in 1874 and by Bruchmann in *Pinus sylvestris* in the same year. It was generally held that the condition of such roots was due to parasitic invasion, and Reess (1880) believed the fungus present in *Pinus* to be *Elaphomyces granulatus*, the False Truffle or Hirschtruffle, the fruits of which he had found in quantity on roots of this tree. Boudier (1876) had already published a paper suggesting the probability of a parasitic habit in certain species of the genus *Elaphomyces*. Kamienski accepted this view, and although positive evidence for the identity of the root fungi of *Monotropa* with the genus *Elaphomyces* was lacking, believed that the same or a similar fungus was parasitic upon the distorted roots of Pine and Beech found interlaced among those of *Monotropa*. In a paper published in 1886, he stated his conviction that, in the mycorrhizas of trees, the roots were subject to attack by parasitic fungi without deriving any benefit from the latter; the mycorrhiza of *Monotropa*, on the contrary, he held to be a symbiotic association in which nutriment was conveyed to its vascular host by a non-parasitic fungus.

Lacking experimental evidence as to the behaviour of the vascular plant in the absence of its root fungus, Kamienski's views concerning nutrition belong to the region of speculation rather than to that of scientific fact. Inasmuch as the results of experimental research on

this subject are not yet available, they are still of theoretical interest and may be criticised in the light of recent experiments on analogous cases. The hypothesis advanced to explain the nutritive relations in *Monotropia* was as follows. The plant grows in soil rich in humus and absorbs the whole of its food supply from the soil by means of the roots. Owing to the presence of a thick and continuous sheath of mycelium, the absorbing surface of the roots has no direct contact with the soil, and all soluble food materials entering the plant from that source must pass through the mycelial envelope. No evidence of parasitism on the part of the fungus was found and it was held that the hyphae grew upon the surface of the roots merely as on a convenient base offering a larger surface than the surrounding soil particles. In return for this hospitality the fungus was believed to provide nourishment for its host—"fournit au *Monotropia* de la nourriture." Physiologically, the mycelial mantle was assumed to function as the epidermis of the root, and the hyphae extending outwards from it as root hairs. It was further concluded that the demands made upon the fungus by the vascular plant could not be excessive since the former continued to use the roots rather than the soil as a substratum favourable to growth. With regard to the nature of the nutritive materials, it was held that both fungus and vascular plant could utilise the organic compounds present in humus, but Kamienski also put forward a view—believed by him to be strongly supported by the observational facts,—namely, that the mycelium on the roots of *Monotropia* was continuous with that growing *parasitically* in the roots of the neighbouring trees. This hypothesis rested upon the assumption that the mycelium upon the roots of *Monotropia* was identical and continuous with that in and upon the roots of Pine and Beech. Its acceptance or otherwise did not affect his general view as to the reciprocal relation existing between the two symbionts in the case of the former plant. "Cette relation étrange entre le champignon et le *Monotropia* n'est pas un fait unique et isolé dans la nature. Nous pouvons le ranger avec d'autres faits semblables auxquels M. de Bary a donné la dénomination de 'symbiose.'"

The fundamental weakness of the view put forward by Kamienski—as was undoubtedly realised by himself—was the complete lack of experimental evidence showing the dependence or otherwise of the *Monotropia* plant upon its fungal partner. Could the vascular plant absorb the necessary organic matter from the soil humus without the intervention of its root fungus? There is a lack of

precision in the statements bearing on this that has remained characteristic of the subject as presented in botanical text books ever since. On the one hand, it is definitely stated that the roots have no direct contact with the soil, on the other hand, it is implied that the mode of nutrition of each of the partners is similar in respect to the utilisation and absorption of organic compounds in the soil. To accept the view that a fungus can utilise the humus constituents of soil and at the same time grow parasitically upon roots of various trees presents less difficulty to the modern botanist than it did to Kamienski. It is more difficult to make the assumptions demanded by his hypothesis that mycelium of one and the same species can simultaneously grow *saprophytically* in the soil, *parasitically* upon roots of Beech and *symbiotically* upon roots of *Monotropa*. Kamienski obviously inclined to the view that the vascular plant profited from the association, although he evaded the consequences involved by this hypothesis, i.e. that *Monotropa* is directly parasitic on the fungus and thus indirectly parasitic upon the roots of the surrounding trees.

In all modern botanical textbooks, *Monotropa* is classed as a saprophyte. Whether this is true in the strict meaning of the word, or in exactly in what sense it is to be understood, remains as uncertain now as when Kamienski published his paper in 1882. Nor can any positive statement yet be made regarding the possible identity of the root fungus or fungi of *Monotropa* with those present in what was afterwards called the ectotrophic mycorrhiza of Pine and Beech.

On the other hand, the identity of the fungi concerned in the formation of mycorrhiza in *Pinus* is no longer a matter of surmise (see Chap. VI), although that of the forms present in Beech has not yet been established with certainty.

Kamienski's memoir on *Monotropa* has been considered in some detail because his work has been somewhat unfairly overshadowed in the later literature by that of Frank. There can be no doubt that the former first recognised and stated the existence of a reciprocal relation between flowering plant and fungus in *Monotropa*. This claim was emphatically put forward at the time by Woronin (1885), who, after the publication of Frank's first paper, concluded a somewhat fiery criticism as follows:—"Alle Prioritätsrechte in der Frage über die auf Wurzelsymbiose beruhende Ernährung gewisser Bäume durchunterirdische Pilz müssen demnach nicht Herrn B. Frank, sondern Herrn Fr. Kamienski zugeschrieben werden."

As a matter of fact, priority in this matter as a whole belongs neither to Kamienski nor to Frank, but to Pfeffer (1877), who

ascribed to the Orchid fungi a physiological rôle analogous to that of root hairs. His views on the physiology of the relationship in Orchids were clearly stated as follows:

Hier (bei *Neottia*) kann man in der That nicht umhin anzunehmen, dass eine Association vorliegt, aus welcher auch die bewirthende *Orchideen* Nutzen zieht, indem sie von dem parasitisch und saprophytisch lebenden Pilze Nährstoffe empfängt, welche die Pilzfäden aus den Boden aufnehmen....Ich bin zwar überzeugt dass die *Orchideen* auch ohne solche Pilze bestehen können, indess ist damit nicht ausgeschlossen, dass die Pilzfäden, da wo sie vorhanden sind, ihrem Wirthe Nutzen bringen.

Although the justice of Frank's claim to priority is open to question, the importance of his work on the subject needs no emphasis. Indeed, the scanty space assigned to mycorrhiza in botanical text books is occupied chiefly with the results of his observations and with speculations upon their significance. His first paper in April 1885 was quickly followed by a number of others in which he extended and co-ordinated his earlier observations. Not only did he carry out extensive observations himself but his example stimulated many other workers to research in the same field, as is testified by the large literature contributed subsequently by his colleagues and students.

The investigations carried out by Frank were undertaken in the first instance at the request of the German State Forestry Department in connection with the proposed cultivation of truffles in Prussia. They had as a starting point the observed association of truffles with certain trees, especially with Beech, Hornbeam, and Oak, in conjunction with the researches of Reess (1880), who had described what he regarded as a parasitic infection of the roots of *Pinus sylvestris*, the mycelium responsible for which he believed to be that of *Elaphomyces granulatus*, fructifications of which were abundant among the same roots. Suspicion was thus aroused as to a possible parasitic relation of the mycelium of truffles with the living roots of trees, and Frank's original investigation was designed to test this possibility. As an immediate result of the work he reported the invariable occurrence of fungus infection in the roots of certain trees, especially members of Cupuliferae, such infection being, in his opinion, not parasitic but of definite service to the trees in relation to the absorption of water and mineral salts from the soil.

Frank regarded this regular association of root tissues and mycelium as a morphologically distinct organ for which he proposed

the name *Pilzwurzel* or *Mycorrhiza*. He recorded the characteristic coralloid growth shown by the mycorrhizas of Cupuliferae and described their structure in detail. He found that mycorrhiza occurred whether truffles were present or not but did not on this account reject the view that *Elaphomyces* or some other member of Tuberaceae was the fungus species concerned, since it was not unusual for mycelium to remain sterile over long periods of growth and special conditions might be necessary for the production of fructifications.

He assumed that infection took place from the soil, and carried out experiments with water cultures in order to test the behaviour of young trees when grown without their root fungus. By this means, three year old seedlings of Oak were obtained showing a well-developed root system without fungus infection. If seedlings possessing mycorrhiza were transferred from soil to culture solutions, they showed a gradual reduction of infection in the newly formed roots. From such experiments Frank concluded that the root fungi could best perform their beneficent service to the trees when growing in soil, although no satisfactory evidence was produced that the growth of the trees concerned was deleteriously affected when they were absent from the rooting medium.

Frank emphasised the close analogy between the thallus of a Lichen and mycorrhiza, each with its green and non-green constituent, and concluded that a true symbiosis existed in roots of Cupuliferae. With regard to the distribution of mycorrhiza he found it to be invariable in Oak, Beech, and Hornbeam, occurring quite independently of soil or situation. He recorded it also in certain species of Willow, and among Conifers in Pine, Fir, and Silver Fir. On the other hand, he gave in the paper under review a long list of native shrubs and trees which did not form mycorrhiza, including among them Birch (*Betula alba*) and Lime (*Tilia europaea*), both of which were afterwards recognised as typical mycorrhiza trees. He also commented on the absence of mycorrhiza from the ground flora of Beech woods, e.g. from roots of *Mercurialis perennis*, *Anemone nemorosa*, and other species in which typical mycorrhiza of a rather different kind from that in Beech has since been described.

On grounds such as these the earlier papers of Frank are open to criticism, as are also, in the light of modern experimental research, his provisional conclusions drawn from experiments. In the history of mycorrhiza the paper published in 1887 possesses a special interest as the first generalised account of a phenomenon now known to be even more widespread than was believed by Frank, and also as

that registering the origin of the term *mycorrhiza*. The energy displayed by Frank in collecting and correlating evidence of the wide distribution of root infection and the surprising character of the facts he brought to light, provide ample excuse for the inclusion of minor inaccuracies. At this stage of the enquiry it was perhaps inevitable that the author's zeal should tempt him to make the facts fit his hypothesis rather than the hypothesis fit the facts.

The outstanding results of Frank's preliminary investigations were, firstly, the recognition of root infection as a widespread phenomenon in trees, and the bestowal upon it of a distinctive name marking its existence as a morphological entity; and, secondly, the rejection of the accepted view of parasitic invasion of these roots, whether by Truffles or other soil fungi, and the substitution of his theory of a symbiotic relationship beneficial to the trees. The far-reaching character of this hypothesis was a direct incentive towards the collection and interpretation of new facts bearing on the subject.

Later in the same year Frank (1885 b) published another contribution to the subject, provoked doubtless by Woronin's claim of priority for Kamienski. While tacitly admitting the latter's claim to priority of observation of root infection, Frank quite justly pointed out that the root fungi were believed to be parasitic on trees and assumed priority for his own interpretation of the association as a phenomenon of mutualistic symbiosis. His conclusions as to the physiological relationship existing in Cupuliferae were concisely stated in this paper and may be briefly summarised as follows.

Mycorrhiza is a symbiotic relation to which probably all trees under certain conditions are subject. It is formed only on soils containing humus or abundant plant remains, and its formation waxes and wanes with the abundance or otherwise of these constituents in soil. The root fungi carry to the trees not only the necessary water and salts but also soluble organic material derived from the humus, thus lending a new significance to leaf-fall and the accumulation of humus in woodland soils, and reviving incidentally an old theory concerning the nutrition of green plants in a sense somewhat different from that in which it was stated originally. The assistance thus rendered to the trees possesses special importance when rapid growth makes heavy demands upon available food constituents in the soil, and is likewise of great significance to non-chlorophyllous species.

During the two years following, Frank extended his observations in many directions and learned that the regular association of fungi

with the roots of vascular plants may show structural characters very different from those found in Cupuliferae, Conifers, and *Monotropa*, regarded hitherto by him as the normal type. Recognition of this led to the publication of another paper in which his well-known classification of mycorrhiza to *ectotrophic* and *endotrophic* forms was proposed. "Wenn wir alle diejenigen Formen bei denen der ernährende Pilze sich auswendig befindet als *ectotrophische* und diejenigen, wo er das Innere gewisser Wurzelzellen einnimmt als *endotrophische* bezeichnen, so erhalten wir folgende Uebersicht" (Frank 1887).

The descriptive terms endotrophic and ectotrophic have been in general botanical use since that time. The distinction between typical and extreme cases of the two kinds is sufficiently obvious. Owing partly to defective observations and partly no doubt to the great weight attaching to Frank's views on the subject, the essential characters of the distinction between the two types was over-emphasised by some of his contemporaries and successors. The use of the terms in a somewhat rigid sense in botanical textbooks has doubtless delayed the recognition of forms intermediate in structure between the two extreme types. Recent work on the cytology of ectotrophic forms confirms the view that mycorrhiza showing the structural characters of both types is not uncommon and that the presence of intracellular mycelium in the cortical cells of ectotrophic mycorrhiza is relatively frequent—in short, that the difference is one of degree rather than of kind.

The review which accompanied the analysis and classification of types in this paper of Frank's is of interest as a summary of the observations available at that time and merits brief notice.

Ectotrophic Mycorrhiza.

The distinguishing features of the ectotrophic type described by Frank in Cupuliferae, Conifers and other trees were:—(1) the invariable presence of mycorrhiza throughout the life of individual trees from the first year of growth onwards, and its distribution upon the actively absorbing regions of the root system; (2) the coralloid growth exhibited by infected roots or root systems; (3) the absence of root hairs and reduction of the root caps to a few cells only; (4) the presence of a complete investment or mantle of fungal hyphae covering the whole of the younger parts of the roots including the apices; (5) the existence within the root tissues of a continuous network of hyphae separating the individual cells of the epidermal and cortical layers, the fine hyphae composing the network being

continuous with those in the external mantle and not infrequently penetrating the epidermal cells; (6) the absence of intracellular mycelium from the cells of the cortical region.

Frank recognised the variation that existed in the structure of the mantle in regard to thickness, coloration of hyphae, and nature of the surface, e.g. whether smooth or covered with projecting hyphae extending into the surrounding soil. He considered that such differences were largely specific to individual types of mycorrhiza and did not depend upon age or degree of development. Cases were described differing remarkably from what was regarded as the normal type. For example, an anomalous long-branched—i.e. not coralloid—mycorrhiza on Beech from Hanover with a very thick mantle and “pseudo root hairs” was described and figured. The outgrowing strands of hyphae from the surface of this mycorrhiza resembled root hairs both in appearance and in their relation with the soil particles and were believed to function in a manner quite analogous to these organs (Fig. 3). Another peculiar form was recorded upon roots of *Pinus Pinaster* from Capetown. This mycorrhiza, described as resembling a “fox’s brush,” consisted of a main axis, thickly beset with hair-like threads of approximately the same length. Microscopic examination showed the latter to be short, fine lateral roots, each of which was a typical ectotrophic mycorrhiza (Fig. 4 *a, b, c*). This anomalous structure was not found in European material of *Pinus pinaster*.

Endotrophic Mycorrhiza.

Under this heading, Frank described the two types of mycorrhiza characteristic of Orchidaceae and Ericaceae respectively. In view of the fact that considerable space will be devoted to a consideration of these groups in later sections of the present work a very brief summary of the facts noted by Frank will suffice.

The condition of the roots in Orchids had already attracted the attention of a series of observers from the time of Schleiden onwards; of these, a contemporary, Wahrlich (1886), had published a paper dealing especially with the root fungi of a number of Orchid species. The general characters of Orchid mycorrhiza were therefore fairly well known; among the features specially noted by Frank were:—(1) the non-parasitic character of the fungus invasion of the roots and its importance in relation to the nutrition of the Orchid plants; (2) the disposition of the infected tissues in such a position that substances absorbed from the soil must pass through them on

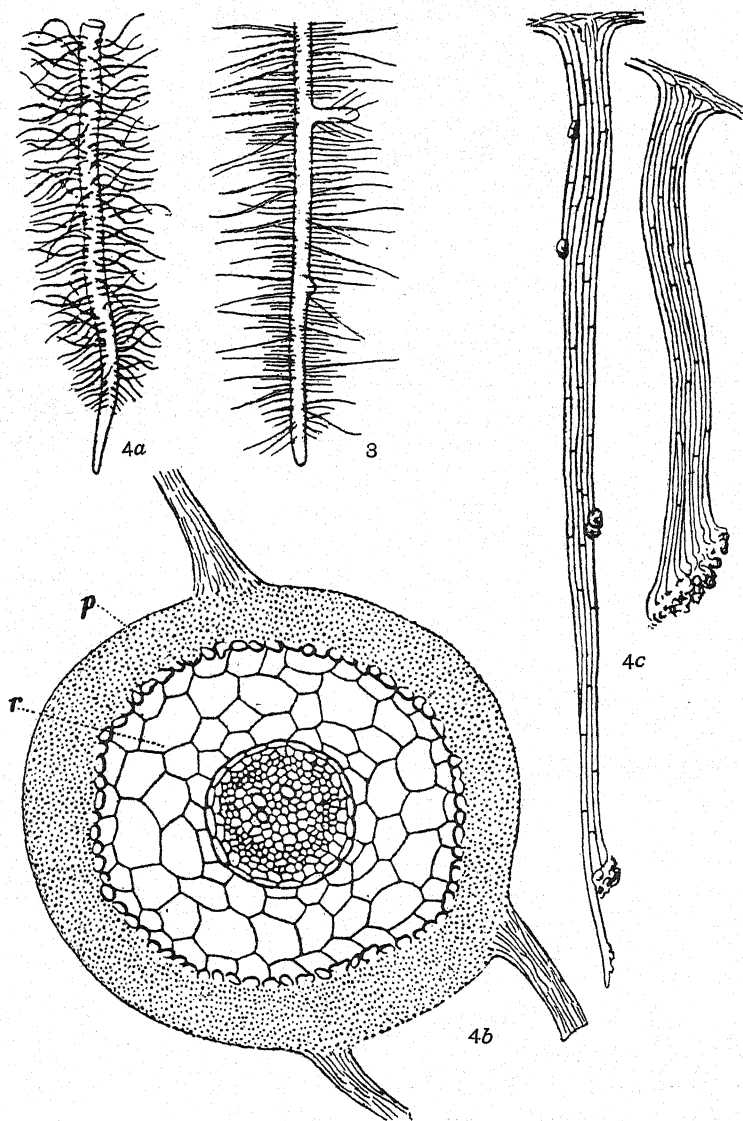


Fig. 3. A mycorrhiza from *Fagus sylvatica* with root-hair-like strands of mycelium. (From Frank, 1887.)

Fig. 4a. Root of *Pinus pinaster* from S. Africa with root-hair-like mycorrhiza. (From Frank, 1887.)

Fig. 4b. Transverse section of the mycorrhiza shown in Fig. 2: *p*, thick fungus mantle with outgoing strands of mycelium; *r*, cortex of root. (From Frank, 1887.)

Fig. 4c. Two mycelial strands from the same mycorrhiza; to show the intimate relation with soil humus. (From Frank, 1887.)

entering the plant, and (3) the invariable presence and high degree of development of mycorrhiza in the non-chlorophyllous Orchids.

In respect to Ericaceae, external infection of the roots of *Andromeda polifolia* had been noted by Frank in 1885, and Thomas (1885) had recorded root infection in Bilberry and inaccurately described it as of similar type to that found in *Monotropa* and members of the Cupuliferae. Subsequently, Frank examined the roots of a number of other members of the family and contributed the first general account of mycorrhiza in the group in the paper under review.

Among the species mentioned by him are *Andromeda polifolia*, *Ledum palustre*, *Calluna vulgaris*, *Rhododendron ponticum*, *Azalea indica*, and a number of species of the genus *Vaccinium*, e.g. *Vaccinium oxycoccus*, *Vaccinium myrtillus*, *Vaccinium vitis idaea* and *Vaccinium macrocarpum*, all of which showed regular and characteristic infection of similar type. The general appearance of mycorrhiza in the fine roots of Ericaceae was admirably described by Frank. Attention was directed to the fact that in Ericaceae, as in Cupuliferae, the formation of mycorrhiza was independent of soil conditions *other than the presence of abundant humus*; e.g. the presence of a thin surface layer rich in humus was noted on sandy heaths carrying ericaceous vegetation. Among outlying members of the group, *Pyrola* was recorded as free from root infection and *Monotropa* noted as differing in the possession of mycorrhiza of the ectotrophic type.

Although not a member of Ericaceae, *Empetrum nigrum* was also included in this account of mycorrhiza in heath and moorland plants as a species possessing root structure and mycorrhiza resembling that found in the Heath family, with various members of which it is frequently associated in the field. With the exception of *Empetrum* and Ericaceae, mycorrhiza was not recognised by Frank in other members of moor and heath vegetation in North Germany; for example he recorded it as absent from the roots of *Aspidium thelypteris*, *Carex vulgaris*, *Drosera rotundifolia*, *Viola palustris*, *Menyanthes trifoliata*, *Comarum palustre*, *Tormentilla erecta*, and *Trifolium repens*.

Observations on mycorrhiza in Germany were confirmed by examination of material sent from other countries and by the accounts of independent observers. Thus, records from S. Africa for Cupuliferae, Conifers, and Heaths, from Australia for *Fagus Cunninghamii*, and from New Holland for Epacridaceae, gave evidence of its wide distribution, and confirmed the view expressed by Frank

as to the invariable occurrence of mycorrhiza in certain species however wide their geographical range. Möller confirmed Frank's observations on Beech for Danish material and Ascherson recorded the presence of mycorrhiza in roots of Alpine species of Willow (*Salix retusa* and *Salix reticulata*) up to 3000 metres.

The later publications of Frank (1888-1894) are concerned especially with the elaboration of his theory of nutrition in mycorrhiza plants, and the accumulation of experimental evidence in support of it. In their more general aspects these views have become an integral part of the literature of the subject. The observations and experiments on which they were founded are not so well known to modern botanists; in view of the fact that almost 40 years have elapsed since their publication and that they serve as a convenient starting point for a survey of recent work on the subject in a later section of the present review, the following brief recapitulation may serve a useful purpose.

Frank believed that the existence of the appropriate root fungi in soil—and hence the formation of mycorrhiza by trees—was conditioned by the presence in woodland soils of humus derived from fallen leaves and other organic residues of the trees growing in such soils. This belief he tested experimentally by comparative observations on Beech seedlings raised upon soils with and without humus, or transferred from their natural habitat in woodland soils to pots of sand. In general, the results of such experiments were interpreted as favouring the view that the root fungi were present in woodland soils and depended upon the humus present for their nutrition.

That humus from other sources was not necessarily effective for the purpose was deduced from experimental cultures; thus, in humus soils other than those of woodland origin, Beech seedlings were observed to grow unsatisfactorily, and failed to develop mycorrhiza. Similar conclusions followed from transplanting young Beeches from natural woodland soils to others lacking humus. The transplantations were successful, the seedlings established themselves afresh, but the new roots present at the end of two years were almost entirely deprived of mycorrhiza.

It was concluded by Frank that the root fungi of trees tended to disappear when humus of the appropriate kind was absent, i.e. that their proper nutrition depended upon an adequate supply of this soil constituent. In his own words:—"die Mykorrhizapilzen nicht in den lebenden Pflanzenwurzel ihre Lebensbedingungen finden, sondern vielmehr in gewissen Beschaffenheiten des Bodens

und dass es vor allen Dingen der Baumhumus ist, von welchem die Anwesenheit dieser Pilze und der von ihnen gebildeten Mykorrhizen abhängt."

His further conclusions as to the relation of the root fungi of trees to the nutrition of their hosts were based mainly upon the structure of ectotrophic mycorrhizas, the correctness of the deductions being tested experimentally by sand and water cultures of Oak and Beech. The general character of these conclusions is familiar to all botanists and may be summarised briefly as follows.

The fungus mantle with its outwardly extending hyphae acts as the absorbing system of the tree, through which water and mineral salts must pass on their way to the vascular strands. The hyphae of the inner part of the mantle and those forming the internal network are continuous with those outside and are so placed that osmotic exchanges can take place with the epidermal and cortical cells with which they are in contact.

The existence of a similar type of mycorrhiza in *Monotropa*, a non-chlorophyllous species, in conjunction with the results of experiments designed to test the effect upon nutrition of withholding a supply of humus from Beech seedlings, led Frank to include the formation and intake of suitable compounds of carbon and nitrogen from humus among the services rendered by the root fungi to their hosts. Experiments were carried out to obtain evidence in support of this view. Thus, it was found that Beech seedlings germinated in sand or water cultures and, supplied with the necessary inorganic salts, did not form mycorrhiza, grew badly and died after one year's growth. Similarly, when parallel cultures of Beech were grown, using woodland soil untreated and the same soil sterilised at 100° C., all the seedlings planted in the former developed typical mycorrhiza and grew vigorously, while those raised in sterilised soil formed no mycorrhiza, died off one by one, and had all succumbed at the end of three years.

The experimental evidence was believed to prove that both the Beech and its specific root fungi required a supply of humus for their proper nutrition and could make but scanty growth if this was lacking. Deprived of mycorrhiza, Beech seedlings suffered inhibition of growth and eventually died because they were unable to utilise the food supplies locked up in humus. In other words, the trees depended upon their fungus symbionts for the re-conversion of their cast off residues into active plant substance. In a subsequent paper, similar experimental results were recorded for Pine (*P. sylvestris*),

using comparative seed cultures in untreated soil from a Pine forest and similar soil steam-sterilised for one hour at 100° C. The seedlings in the untreated soil behaved as in seed-beds under natural conditions; those in the sterilised soil made poor growth, showed reduced size and discoloration of the needles and at the end of two years were in a moribund condition. The former developed typical mycorrhiza; the condition of the latter in respect to this is described as follows:—"die mikroskopische Prüfung der Wurzeln ergab aber keine Spur von Verpilzung, die Wurzeln kurze Wurzelhaare gebildet."

Since it had been shown previously that plants other than those with mycorrhiza grew well in sterilised soil, it was concluded that the roots of Conifers, like those of members of Cupuliferae, no longer function like the roots of ordinary plants but depend for their proper nutrition upon the presence of their appropriate root fungi.

Inasmuch as this was the case in trees and in *Monotropa*, these plants must be regarded as *parasites upon saprophytic fungi*. Thus:—"Insofern also der Baum seine Nahrung unmittelbar aus der Hand des Pilzes und erst durch dessen Vermittelung aus dem Erdboden empfängt musste man vielmehr die Waldbäume und die in den Wurzeln sich dessen gleich verhaltende *Monotropa hypopitys* als Parasiten auf saprophyten Pilzen betrachten." No ordinary parasitism this, but "eine mutualistisches Verhältniss, eine wirkliche Symbiose"!

These views were not endorsed by Henschel (1887) for the mycorrhiza of Conifers. On the contrary, the observations made by this worker led him to the view—"dass der Einfluss diesen Symbioten auf der Entwicklung der jungen Fichtenpflanze als ein absolut schädlichen angesehen werden müsse."

The belief that woodland humus in particular was an important source of nitrogenous food material was greatly strengthened by independent observations on the nitrate content of trees and other mycorrhiza plants on various soils. Frank and Molisch had previously shown that a direct relation existed between deficiency of nitrates in soils and a similar deficiency in the tissues of plants growing upon them, from which it was reasonably inferred that vascular plants were unable to convert either free nitrogen or compounds of nitrogen into nitrates. Moreover, Frank (1887) had recorded the absence of nitrates from the aerial parts of various trees and their absence, or very scanty appearance, in the absorbing roots, while the tissues of *Neottia* were found to give no reaction for nitrates although such is usually well-marked in herbaceous plants.

An additional link in the chain of evidence was provided by

Ebermayer (1888), who, in a contemporary paper entitled "Warum enthalten die Waldbäume kein Nitrate?" recorded the almost complete absence of nitrates from more than one hundred samples of woodland and moorland soils.

All these facts led independently to the conclusion that plants growing upon soils deficient in nitrates must obtain their supplies of nitrogen either as ammonium salts or as organic compounds of this element. They pointed also to the possible significance of the root fungi in this connection and, assuming likewise the importance of carbon compounds other than carbon dioxide for non-chlorophyllous species such as *Neottia* and *Monotropa*, justified the conclusions expressed by Frank in the following passage:—"bei den chlorophyllhaltigen Pflanzen die Mykorrhizapilze hauptsächlich die Erschliessung des Humusstickstoffes bewirken." His own experiments led him to believe that certain plants, e.g. Oat and Lupin, which were believed free from fungus infection, could directly utilise the organic compounds present in soil. It was pointed out also that potassium and other essential elements were locked up in the organic residues of humus and might be rendered accessible to vascular plants by the metabolic activities of their root fungi.

The theory of nutrition outlined above was extended by Frank to cover cases of endotrophic mycorrhiza, the number of which had been greatly extended by the researches of his pupil Schlicht (1889) and by those of Warhlich (1886) on the Orchids. The failure of many of these plants to produce root hairs was noted; as was also the position of the infected cells in a zone of tissue between the absorbing and conducting regions of the root. Owing to the scanty development of mycelium *outside* the roots, it was impossible to relate the root fungi directly to absorption as in ectotrophic mycorrhizas; moreover it was uncertain to what extent the intracellular mycelium drew upon the root cells for nutritive materials. On the other hand, Frank had recently studied the mycorrhiza cells in certain Orchids—in particular in *Orchis latifolia*—and had been greatly impressed by the extensive digestion of mycelium in these cells and the subsequent removal of the products of digestion as deduced from staining reactions. Here, indeed, was a new and quite unique kind of symbiotic relation between vascular plant and fungus! He extended these researches on Orchids to the fine roots of *Ledum palustre* and *Empetrum nigrum*, which showed similar staining reactions with aniline blue and were described as quite analogous to the Orchids in this respect.

Frank therefore included all the known cases of endotrophic mycorrhiza as typical examples of mutualistic symbiosis, although he deplored the circumstance that experimental proof of the symbiotic relation, in the form of evidence as to the behaviour of infected and uninfected plants in comparative cultures, was still lacking both for Orchids and Heaths.

The evidence of intracellular digestion found in Orchids was compared with that exhibited by insectivorous plants, and the strength of Frank's convictions as to the beneficial rôle of endotrophic mycorrhiza is evidenced by the close analogy that he postulated between the two classes of plants. "Denn der Pilz ist hier gleichsam in den Wurzelzellen gegangen, wo er als sichere Beute zuletzt von der Pflanze aufgezehrt wird."

Although he fully realised and admitted the importance of comparative cultures with and without fungus infection, the experimental evidence offered by him on this fundamental aspect of the subject is unsatisfactory in the case of ectotrophic mycorrhiza, and lacking altogether for the endotrophic forms, with which no experimental work was attempted. In a later chapter of the present review it will be of interest to compare the opinions of Frank, based largely on theoretical considerations, with the results of recent researches, for which pure cultures of certain trees and of their root fungi have been available.

Cogent as are many of the arguments based upon the structure and cytology of mycorrhiza and ably as the evidence is marshalled in these papers, it is difficult to avoid the imputation of special pleading to the part of Frank's work relating to experimental observations. Some, at least, of his results are open to interpretations other than those offered, and the experimental evidence is in itself too slight to carry the imposing edifice raised upon it.

His interpretation of the facts was not always acceptable to contemporary botanists; in particular, his views invited criticism from students of the effects of parasitic invasion by fungi, at that time a new branch of botanical science. In his first paper Frank had referred to the work of Gibelli (1883) on a disease of the Chestnut in Italy, criticising the opinions of the latter with regard to the relation of parasitic fungi believed to be responsible for the pathogenic conditions with the hyphae of the mycelial envelope likewise present on healthy roots. On behalf of Gibelli, Frank's criticisms on this matter were questioned by Penzig (1885) in a subsequent paper.

Hartig (1885) had described a disease of young Oaks attributed

to parasitic attack by a fungus, *Rosellinia* (*Rhizoctinia*) *quercina*, but the condition of the roots showed no agreement with that of the mycorrhiza of Oak described by Frank.

Publication of the latter's theory of symbiotic nutrition in 1885 quickly provoked a criticism from Hartig and Groszick (1886), who put forward the view that mycorrhiza in general is a pathological condition brought about by the attack of parasitic fungi. In reply, Frank emphasised the relative longevity of mycorrhizas as compared with roots subjected to ordinary parasitism, the active growth of the former subsequent to infection, and his experimental demonstration that in humus-free soil the fungi desert the roots instead of parasitising them. The arrest of growth in length and increased branching, noted by Hartig as symptoms of disease, were regarded by Frank as adaptations securing concentration of the absorbing system in those regions of the soil where humus was most abundant. In support of this view, he cited the well-known fact that a rich substratum promoted abundant branching of roots with relatively limited growth in length, whereas one relatively poor in nutrients led to scanty branching with greater elongation.

Hartig (1888) specially emphasised his own observations that young roots of trees were free from fungus infection during the period of maximum absorption from June to September. As they became infected from older roots this functional activity diminished and might cease altogether until a fresh supply of young roots developed in the following spring. Kamienski (1886) also disagreed with Frank's theory of beneficial symbiosis in the case of trees, pointing out that infected roots were less common than reported and that the tissues were often pathological in appearance. He re-stated his own view that the relation in trees was one of parasitism on the part of the fungus, while in *Monotropa* it was one of mutual symbiosis resulting in a supply of nutrient to the host plant. He claimed that the chief merit of Frank's work consisted in drawing the attention of botanists to a phenomenon which invited research and was worthy of greater attention.

The challenge issued by Woronin (1885) in respect to the priority of Kamienski's observations has already been noted. In the same paper this author states that the regular root infection described by Frank had been independently observed by himself in roots of various members of Cupuliferae, Salicaceae and Coniferae, while working on certain edible species of *Boletus*. Woronin did not challenge Frank's interpretation of the facts, beyond stating

his own conviction that the relation was one of parasitism on the part of the root fungi. He pointed out also that in Finland, where many of his own observations had been made, the mycelium present in roots was not likely to be that of Truffle fungi since the latter were not found in that country.

Papers dealing with the same subject, i.e. the parasitism of Truffle fungi and their relation to mycorrhiza, were also published by Müller (1886) and by Mattiolo (1887); the latter reached the provisional conclusion that a parasitic mycelial investment of the roots of certain trees—"rizomorfa parassite delle radici"—in all respects similar to those described by Frank under the name of mycorrhiza, could, under conditions not yet fully ascertained, give rise to the reproductive bodies of *Tuber excavatum*.

The identity of the root fungi was still a subject of controversy. Tulasne (1856) had noted that *Elaphomyces* formed a coating upon roots of Pine regarded by him as of parasitic nature. Later (1862), he modified this view and expressed the opinion that the two constituents derived mutual benefit from the association.

Reference has been made to the work of Reess (1880) who had suggested that the mycelium in mycorrhiza was that of *Elaphomyces granulatus*, a fungus believed to be related to the Truffles. His further researches (Reess 1885, 1887) had demonstrated the striking similarity of the mycelium in roots of *Monotropa*, Conifers and members of Cupuliferae, but provided no positive proof of its identity with that of *Elaphomyces*; moreover, they introduced the difficulty of knowing whether one or a number of fungal species were concerned in the formation of mycorrhiza. In Pine, for example, upon the roots of which spore bodies of this genus had been found, there was evidence of the presence of another type of mycelium resembling that characteristic of Basidiomycetes. It is clear from his later papers that Reess had an open mind on this aspect of the subject, and, without rejecting the opinions expressed by Kamienski and Frank with respect to nutrition, believed that further evidence was required. Thus, as arguments against the symbiotic view he cited, (1) the absence of mycelium from the roots of many woody plants, (2) the occasional abundance of root hairs in mycorrhiza plants, e.g. in Pine, and finally, (3) the numerical relation between infected and uninfected roots in Pine. While respecting Frank's views on the subject it was clear that he did not agree with them.

Referring to the paper by Woronin on this subject, Lecomte (1887), recording his own observations in the Vosges, stated that conidia

and perithecia observed by him on roots of Hazel were produced by the mycorrhizal fungus specific to the tree; the reproductive structures observed were believed to indicate affinity with members of Perisporiaceae. In the same paper reference was made to similar observations by an Italian mycologist, Cornu, in conjunction with Gibelli (*loc. cit.*), who also held that the mycelium in roots of Cupuliferae, Conifers, etc., belonged to species of *Elaphomyces* or *Hymenogaster*. That other aspects of the subject also attracted attention is evidenced by the observations of P. E. Müller (1903) on the nature of humus and the distribution of fungi growing in it.

Noack (1889) investigated the relationship between the mycelium of various Hymenomycetes and Gasteromycetes and the mycorrhiza of certain trees. For example, he observed that coralloid roots of *Pinus sylvestris* were almost invariably present in the humus below the sporophores of *Geaster fimbriatus*. By similar observations on fruit bodies Noack was led to include the following fungi as mycorrhiza formers: *Geaster fimbriatus* and *Geaster fornicatus* on *Pinus sylvestris* and *Abies excelsa*; *Agaricus* (*Tricholoma*) *russula* on Beech, forming the rose-pink mycorrhiza described by Frank; *Agaricus terreus* on Pine and Beech, *Lactarius piperatus* on Beech and Oak; *Cortinarius* sp. on Fir, Beech and Oak. He noted also that a rose-coloured mycorrhiza on roots of *Pinus sylvestris* was clearly associated with fruit bodies of *Elaphomyces granulatus*. Unfortunately, Noack's conclusions were based almost entirely upon microscopic examination of roots and the propinquity of fruit bodies; attempts to reproduce mycorrhiza from spores of *Geaster* sp. in fungus-free seedlings of Fir,—“die gewiss für die ganze Frage von grosster Wichtigkeit sind,”—gave only one case of doubtful infection.

Mollberg (1884) and Wahrlich (1886) had made further contributions to the already extensive literature dealing with the mycorrhiza of Orchids. The latter writer does not refer to Frank's work and throughout alludes to the root fungi of the Orchids as parasites (Wurzelparasite). His researches sought to determine the real nature of the yellowish slime masses in the root cells, to learn whether a single fungus species was responsible for infection throughout the group, and to observe the mode of fructification of the endophytes with a view to their proper classification.

Examination of a number of European Orchids and of some 500 exotic species showed that the roots of all were more or less heavily infected by mycelium of the same general type. Microchemical investigation of the intracellular slime masses described

by previous observers confirmed the view that they consisted of tightly wound hyphae embedded in an oily or resinous material believed by Wahrlich to be produced by the mycelium. He was not so successful in his efforts to cultivate and identify the endophytes. The technique adopted was crude, and mycelium from a number of Orchid species when cultivated outside the plants gave rise to spores of the *Fusiformium* type described earlier by Reissek and by Schacht. The fungi isolated from several species of *Vanda* produced perithecia, thus leading Wahrlich to place them in the genus *Nectria*. In his view, the general similarity of structure in the mycelium associated with roots pointed to the inclusion of all the root fungi in one generic group, minor differences in the structure of the mycelium and spores suggesting that this comprised more than one species.

Attempts to cause re-infection of young aerial roots by means of spores and thus establish the identity of the forms isolated were unsuccessful, in spite of which Wahrlich committed himself to the following conclusions:

Fruchtificationsorgane des Wurzelparasiten der Orchideen sind *Fusiformium*sporen, Megalosporen und die bei *V. suavis* und *V. tricolor* beobachteten Perithechien mit Ascosporen....Nach seinen Fruchtificationsorganen ist der Pilz als ein *Pyrenomycet* zu bezeichnen. ...Auf Grund dieser Merkmale sind die in *Vanda* Wurzeln parasitirenden Pilze als *Nectria*arten zu bezeichnen....

This paper by Wahrlich is frequently quoted, although it contributed little new to the contemporary knowledge of Orchid mycorrhiza. The major part is devoted to an account of the attempts made by the author to isolate the root fungi from various Orchids and to cultivate them outside the plant; the conclusions reached were entirely uncritical and were unsupported by any experimental proof of the identity of the fungi isolated with the true endophytes.

Schlicht (1889), working in Frank's laboratory, published an account of his researches on endotrophic mycorrhiza in herbaceous plants. He described in detail that found in *Paris quadrifolia*, *Ranunculus acris*, *Holcus lanatus* and *Leontodon autumnalis*, noting its restriction to the actively absorbing roots and the position of the infected tissue between the absorbing and conducting regions. He observed, moreover, that mycorrhizas not uncommonly persisted as main roots, and that the presence of intracellular mycelium offered no impediment to growth, thus controverting the conclusions of Hartig with respect to parasitism.

The possession of this "neuen Mykorhizen" was recorded by

Schlicht for some 112 herbaceous species distributed over about 45 families of Angiosperms, and it was concluded "dass die Symbiose zwischen Wurzeln und Pilzen eine ungeahnte Verbreitung über unsere Flora hat." Observations such as that recording complete absence of root mycelium from certain groups, e.g. Gentianaceae, certainly required revision, as did also the list compiled by Schlicht of mycorrhiza-free species belonging to 22 families. Reviewing the published work of Kamienski, Reess and Frank on the subject, Schlicht pointed out that the relation involved parasitism on the part of either vascular plant or fungus, or a condition of mutualistic symbiosis. In view of his association with Frank, it is perhaps not surprising to find that his own view favoured the last alternative:—

dass die von Frank aufgestellten Thesen über das Wesen und die Bedeutung der Mykorrhizen der Bäume durch die nunmehr erkannte Verbreitung der endotrophischen Mykorrhiza eine noch viel weitere Ausdehnung erlangt haben, denn aus meinen Beobachtungen ergibt sich das überraschende Resultat, dass auch die chlorophyllführenden Kräuter im Allgemeinen nicht ohne einen Wurzelpilz auftreten, und dass auch sie anscheinend nicht ohne diesen bestehen können, wenn ihnen nicht die aus dem Boden aufzunehmende Nahrung in einer anderen ihnen zusagenden Form geboten wird.

CHAPTER III

Humus plants—Saprophytes and hemisaprophytes: Höveler—Johow—Groom—Janse—Hesselman—MacDougal—The cytology of intracellular digestion: Magnus—Shibata.

1890–1900¹.

The recognition of root infection as a regular and widespread phenomenon, and the attention attracted to the physiology of the relationship by the new theory of beneficial symbiosis, stimulated investigation in the subject during the closing years of the nineteenth century, more especially in relation to the study of the endotrophic type of mycorrhiza.

Some of the researches, e.g. those of Schlicht, mentioned above, were carried out in Frank's laboratory, and indeed many of those published during the earlier part of the period were obviously influenced by his point of view and directed to extend and confirm his conclusions. During the closing years of the nineteenth and the

¹ Frank's *Lehrbuch der Botanik* was published in 1892, and included a summary of his observations on mycorrhiza and his views respecting its significance.

first five years of the twentieth century the cytology of the infected root cells specially attracted attention, and knowledge of various aspects of this part of the subject was extended by the researches of a number of English and continental botanists. Many of the observations published about this time were made upon Orchids which afforded exceptionally favourable material for the study of the progressive changes in the infected root cells. The following survey of the more important papers published during the concluding years of the last century will serve to indicate the quickening interest of botanists in the subject of mycorrhiza and its increased importance as a branch of botanical investigation.

Mention may first be made of the comprehensive review of work on root symbiosis and mycorrhiza published by Sarauw (1892). Numerous facts of historical interest are preserved in this paper, which included an exhaustive and accurate bibliography of the literature of root-infection, parasitic and otherwise.

About the same time appeared a paper by Höveler (1892) dealing with the soil humus as a source of food material for green plants.

The old view as to the importance of humus in this connection had long been discredited, but little was known as to the exact chemical nature of this important soil constituent. The subject had engaged the attention of a number of workers, and numerous publications had already appeared dealing with the origin and nature of the soil humus and the utilisation of organic compounds by green and non-green plants whether by direct absorption, or indirectly, through the agency of root fungi.

The researches of Frank (1888) and Koch (1887) on this subject have been mentioned. Among other contributors to the literature of the subject were Stutzen and Klingenberg (1882), Baumann (1886), Müller (1887, 1889), Deherain (1889), Hoppe-Seyler (1889), Acton (1889), and Berthelet and André (1892).

Höveler devoted special attention to the presence or absence of root hairs and the incidence of fungus infection in humus plants, an aspect of the problem indicated by Frank's insistence on the absence of root hairs from plants with ectotrophic mycorrhiza, and their relative infrequency in those showing the endotrophic type of infection. Höveler recorded abundant root hairs of a rudimentary type in Orchids generally, noting also the presence of endotrophic mycorrhiza in *Epipactis latifolia*, which, in common with *Listera ovata*, Frank had previously described as free from fungus infection. In general his observations lent support to the view that plants

with abundant root hairs were free from root infection, although *Calla palustris* was noted as a species possessing both root hairs and mycorrhiza. Among humus plants producing root hairs but growing under field conditions favourable to the formation of mycorrhiza, *Eriophorum* spp., *Narthecium ossifragum*, *Myrica gale*, *Pinguicula vulgaris*, *Hydrocotyle vulgaris*, and *Drosera* spp. were cited as entirely free from fungus infection. In the light of modern researches this list of "fungus-free" species is a useful illustration of the need for an improved technique in investigations of this kind. The author confirmed Frank's records as to the absence of root hairs in Ericaceae, observing as a noteworthy fact that members of this group formed mycorrhiza even when growing in sandy soil deficient in humus.

Höveler sought experimental proof that many plants living on humus soils could use the organic compounds present, but his researches were inconclusive in respect to this and were not confirmed by contemporary workers.

Later, Reintzer (1900) and Nikitinsky (1902) carried out investigations on the decomposition and utilisation of humus, but their results threw little light upon the nutritive relations in mycorrhiza plants.

The attention attracted to *Neottia* and other non-chlorophyllous species in respect to their mode of nutrition and the possession of mycorrhiza has already been noted.

Further contributions to the literature dealing with these so-called saprophytes were made by Johow in 1885 and 1889, and by Groom in 1894 and 1895.

Johow's admirable papers on "holosaprophytes," although they dealt but briefly with the mycorrhiza of the species described, had an important indirect bearing on the subject by reason of the special significance of the nutritive relations in the case of vascular plants lacking chlorophyll. The first paper dealt with the anatomy and embryology of a number of holosaprophytic species collected by the author in Venezuela and the Lesser Antilles; the second attempted a comprehensive account of the whole group from a biological standpoint and included descriptions of many species previously imperfectly known, together with a review of all the knowledge then available concerning these curious plants (Plate I, Figs. 5-10).

The use of the term holosaprophyte for non-green vascular species other than obvious parasites is sanctioned by custom and need not now be discussed. As used by Johow, the term was applied to non-green vascular plants growing in a substratum rich in humus.

At the time when these observations were made, practically nothing was known concerning nutrition in these plants, although certain conclusions were inferred from their usual habitat in humus-rich soils and from the fact that those investigated showed regular and characteristic fungus infections of the subterranean organs. Information as to seed germination was fragmentary and unsatisfactory, nothing was known as to their behaviour when grown under strictly controlled conditions, as to the time and manner of fungus infection or the possibility of raising seedlings free from mycorrhiza. It may be added that the field of experimental enquiry in respect to these matters still remains almost unexplored.

Only in the case of one genus mentioned by Johow, viz. *Gastrodia*, has subsequent work thrown light upon the actual method of nutrition and illuminated the nature of the "symbiotic" relation between the vascular and non-vascular constituents.

Non-chlorophyllous species other than parasites were recorded by Johow in 43 genera of Phanerogams—29 monocotyledons, distributed in the three families, Orchidaceae, Burmanniaceae and Triuridaceae, and 14 dicotyledons, all members of Ericales or Gentianaceae. In view of the disparity of numbers between the two groups, it is perhaps significant that the parasitic habit is not known to occur among monocotyledons.

Geographically, the regions richest in species were found to be Malay and equatorial South America. In general, plants were found only in moist shady woods, preferring soils rich in vegetable remains. The majority grew in a loose, spongy substratum of decaying leaves, but certain Orchids—*Epipogum aphyllum*, *Corallorhiza innata* and *Neottia nidus avis*—were also recorded by Johow from clayey soils watered by drainage from humus-rich deposits, and *Hypopitys* was reported exceptionally from grassy places in sand dunes, presumably poor in humus (cf. *Monotropa hypopitys* near Southport, p. 5).

Some of the tropical species grew upon trunks of fallen trees and in similar situations, and a few were epiphytes on living trees. The origin of the humus did not ordinarily appear to be important, but *Hypopitys hypophagea* was specially associated with organic detritus derived from Fir and Beech, and *Dictyostegia orobanchoides* was recorded on a substratum consisting of the dead roots of Palms.

All the species were found to resemble *Neottia* and *Monotropa* in the possession of well-developed subterranean absorbing systems with a scanty development of aerial shoots limited to the flowering axes. The morphology of the absorbing organs showed much

variety in detail, e.g. single tubers or rhizomes with or without roots, rhizomes or roots with "coralloid" branching. Three species of *Galeola*, viz. *G. cassythoides*, *G. foliata* and *G. altissima* are remarkable exceptions, being branched climbing plants which reach a length of 50 to 120 feet.

Anatomical peculiarities were already known in certain genera. The existence of these was confirmed and found to be characteristic of all members of the group. Absence of chlorophyll, replacement of chloroplasts by leucoplasts or chromoplasts, the absence of a main root and the frequent development of the "coralloid" or "bird's nest" type of subterranean organs, the absence or feeble development of root hairs, and finally, the almost invariable development of mycorrhiza, to which Johow, in his first paper had thus alluded:—"Das constante Auftreten eines Parasiten in den Wurzeln dreier Pflanzenarten," these being *Neottia*, *Corallorhiza* and *Monotropa*.

Minor abnormalities of structure appeared in the roots of most species. Scale leaves of various types were borne upon the rhizomes and tubers, and also upon the aerial shoots. The latter were white or yellowish white in colour or showed a marked development of coloured pigment.

Johow recorded the complete absence of stomata as an outstanding peculiarity of shoot structure in these plants, correlation of which with absence of an assimilating and transpiring mechanism being well shown in Burmanniaceae, a group including species with foliage leaves of normal structure. All the species described produced minute seeds with rudimentary embryos.

With one exception, all the saprophytic species known to Johow formed typical mycorrhiza in the roots and (or) rhizomes, endotrophic in the great majority, but ectotrophic in *Monotropa* and its allies. The only exception belonged to the genus *Wulfschlaegelia*, an Orchid closely related to *Neottia*, recorded by Johow as showing no trace of regular fungal infection. In view of the occasional presence of single hyphae mentioned by this author, a re-investigation of the species is greatly to be desired.

The distribution of mycelium in the cortical cells of the roots and rhizomes was described as varying with the species; the cytology of the infected tissues was not investigated by Johow.

In discussing the rôle of the mycorrhizal fungus in nutrition, reference was made by the author to the interesting observations of Schimper on the aerial roots of epiphytic Orchids. As is well known, certain species, e.g. *Isochilus linearis*, have aerial roots of more

than one kind. Observations on those roots which ramified upon the surface of the bark and organic debris showed that fungus infection was confined to the cortical tissues upon the lower side, i.e. to that in contact with the substratum, while the aerial roots which hung freely in the air remained free from fungus infection. This distribution of infection was regarded as evidence that the mycelium was related in some way with the intake of food material—"Durch diesen Befund wird wohl deutlich angezeigt, dass die Mykorrhiza in Beziehung zur Nahrungsaufnahme steht."

Johow accepted the existence of hemisaprophytic species as an established fact, instancing the works of Solms Laubach (1867-1868), Kerner (1887) and Koch (1887) on the subject. As in the case of holosaprophytes, the claim that certain species are hemisaprophytic in nutrition was based on the character of their habitats and upon certain structural features resembling those found in their non-chlorophyllous allies. No experimental evidence was produced that any of these plants could directly utilise the organic residues in humus soils.

In a paper dealing with the same subject Groom (1894) defined saprophytes as "plants which are dependent for their existence on the presence in the substratum of decaying organic matter. Like parasites, they may be divided into those which possess chlorophyll (hemisaprophytes) and those which have none (holosaprophytes)." The scanty experimental evidence for the existence of hemisaprophytes was noted by Groom, however, who pointed out that current views on the subject were largely speculative.

After reviewing the work of earlier observers, including a detailed summary of the conclusions reached by Johow on the subject, the author gave a detailed account of the morphology and histology of four Orchidaceous holosaprophytes from the Indo-Malay region, viz. *Galeola gavanica*, *Aphyllorchis pallida*, *Lecanorchis Malaecensis* and *Epipogon nutans*. Of these previously undescribed species, the three first-named possessed rhizomes with unbranched or feebly branched roots and scale leaves; the last-named species was rootless. In all four species structural features directly related to absorption and to the elimination of excess water from the subterranean organs were described by Groom. In all there was found extensive fungal infection of the roots, and (or) rhizomes, and (or) scales, with a development of endotrophic mycorrhiza similar to that in *Neottia*.

Two other Orchid species, *Corysanthes* sp. and *Spiranthes australis*, were also examined for evidence of hemisaprophytism. Both were

terrestrial plants with foliage leaves and formed endotrophic mycorrhiza. The habit, histology and affinities of the former and the affinities and habitat of the latter were believed to favour the view that both species were hemisaprophytic, i.e. that they utilised the soil humus directly as a source of organic food material. The conclusions reached respecting the relation of fungus infection to metabolism were as follows:—"Essentially connected with the absorbing organs are the mycorrhizal hyphae, which are present in all known saprophytes (except *Wulfschlaegelia*, according to Johow). The function of mycorrhiza is still a mystery. All that is known is that certain plants have on or in their roots mycorrhizal hyphae, that mycorrhiza is absent from roots grown in soils devoid of humus, and that in the case of endotrophic mycorrhiza the fungus does not kill the protoplasm of the cells in which it dwells." It may be noted that this observation was made nearly ten years subsequent to the publication of Frank's theory of beneficial symbiosis. Indeed, in this paper the experimental observations of the latter are dismissed as inadequate and uncritical, although the author, reviewing the distribution of the mycorrhizal fungus in the absorbing organs and its relation with the root cells of these non-chlorophyllous Orchids, believed that his own observations supplied confirmatory evidence for the view "that mycorrhizal hyphae exert a beneficial effect on the host, and that the fungus is not merely a passive companion."

Protolirion paradoxum, a representative of a new genus of monocotyledons described by Groom in 1895, was believed to show affinities with both Liliaceae and Triuridaceae, the root resembling that of members of the latter group in the possession of a well-developed cortex with endotrophic mycorrhiza. The evidence derived from other structural characters was regarded also as indicative of a saprophytic habit.

In 1895 Groom contributed a paper on *Thismia aseroë*. This plant is a member of a genus of curious "holosaprophytic" species belonging to the family Burmanniaceae. It is a native of Malaya and was discovered and first described by Beccari. The plant body consists of a branching system of cylindrical, leafless axes which ramify horizontally in a substratum of decaying leaves. At the flowering period, erect scale-bearing branches bearing terminal flowers arise from the terrestrial axis. The anatomical structure of the creeping axis is anomalous and its exact morphological nature is not evident.

The external and cortical regions of the axis were found to be extensively infected with mycelium, the mycorrhizal tissue showing a high degree of differentiation with regard to the condition of the intracellular mycelium. In the more superficial layers were found slender hyphae with densely staining protoplasm, in the deeper layers (medio-cortex) the cells contained "*conspicuous, dead, yellow, mycelial masses, consisting of portions of distinct hyphae which are connected by slender portions of defunct hyphae with one another.*" Between these two regions was a single layer of cells containing "very slender hyphae, often spirally twisted, which suddenly swell out into intercalary bladder-like bodies often filled with densely stainable protoplasm."

In certain tissues the cytology of the infected cells was peculiar and showed a remarkable development of intercalary hyphal swellings in which degenerative changes ultimately took place. In such cells starch disappeared after penetration by the fungus and did not reappear.

The physiological significance of these facts is very fully discussed by the author, who concluded from comparative observations that there was an "interchange of material between Fungus and host, and that the material is manufactured by the two symbionts respectively."

Frank's designation of cells showing degenerating mycelium in the endotrophic mycorrhiza of Orchids as "Pilzfallen" and of the plants possessing them as "pilzverdauende Pflanzen" was severely handled by Groom in this paper on the grounds that the fungus demonstrably abstracted carbonaceous food from the plant cells in the early stage of infection, and that no *direct* evidence existed that it provided compensation in the form of protein material for the vascular host. "Although the hyphae of endotrophic mycorrhiza in the medio-cortex die soon, the root (or rhizome) cannot be said to act like the digestive organs of an insectivorous plant, because the protoplasm of the hyphae is manufactured partly at the expense and through the agency of the host." Moreover, the invading organism is not killed, indeed there was some evidence that in *Thismia* the hyphae in the outer cortical tissues "acted as haustoria for those outside."

Frank's views as to the different physiological significance of endotrophic as compared with ectotrophic mycorrhiza were also criticised by Groom, who concluded that "the distribution of the two forms of mycorrhiza and the occurrence of transition stages between

their extreme forms, militates against the view that the physiological significance is not the same in both."

Of the alternative views that mycorrhiza is "a highly adapted and symbiotic community beneficial to both symbionts" or a "pure matter of infection of a plant by a Fungus," with a constant struggle between host and tentative parasite—Groom held that the weight of evidence was on the side of the former. His own observations on *Thismia* pointed to the absorption of carbohydrates by the fungus with some compensating return in the form of organic compounds of nitrogen from the humus made available to the host-plant. His observations pointed to a similarity of functions in ectotrophic and endotrophic mycorrhizas and provided no support for Frank's hypothesis in respect to this.

The account of *Sarcodes sanguinea* published by Oliver (1890) served to emphasise once more the remarkable biological features shown by members of the Monotropeae. This curious non-chlorophyllous species had been collected in Pine woods in two localities in the mountains of California. As described by Oliver the vegetative organs of the plant are represented only by a mass of coralloid roots, from which at flowering arises a massive inflorescence axis about 35 centimetres high, closely invested by fleshy scales. As in other members of the Monotropeae chlorophyll is not formed, but the aerial parts of the plant are coloured brilliantly owing to the presence of a soluble red pigment. The root system consists of a brittle mass of densely branched fleshy roots, each lateral member arising exogenously upon the parent root. All the roots develop typical ectotrophic mycorrhiza resembling, except in minor details, that described for *Monotropa*. The apex of each root is enveloped by a sheath of closely interwoven hyphae that extends backwards over the whole surface of the root. Hyphae from the inner part of this fungal sheath penetrate between the epidermal cells of the root but do not enter them.

Members of the genus *Corallorhiza* had long attracted the attention of botanists by reason of their peculiar coralloid rhizomes, absence of roots, and complete or almost complete lack of chlorophyll. The genus contains twelve species widely distributed throughout Europe, Asia, the United States and Mexico. All are brownish or yellowish herbs of similar habit to *Neottia* and *Monotropa*, entirely lacking chlorophyll or developing only traces of that pigment in the later stages of growth. The observations made by earlier workers have been mentioned.

A short paper by Thomas (1893) dealing more especially with *C. multiflora*, a species in which the aerial parts may reach a height of eighteen inches, was followed by one from Jennings and Hanna (1898) on *Corallorhiza innata*. Both papers described the curious trichome-bearing papillae upon the surface of the rhizome of this Orchid, Thomas regarding the hairs as organs of attachment that showed little evidence of any parasitic function, Jennings and Hanna interpreting them as "fungus traps" facilitating infection of the rhizome by the mycorrhizal fungus.

The cytology of the mycorrhiza cells is inadequately described in both papers, neither of which made a large contribution to the knowledge of endotrophic mycorrhiza in non-green plants. In respect to the biological habit, Thomas rejected the hypothesis of parasitism—"instead of being a root parasite as has been supposed, the plant depends chiefly on the symbiotic condition for its food and this is taken by the hyphae from the decaying vegetable matter about." Jennings and Hanna accepted the view put forward by Frank that "the fungus is a living organism captured for the benefit of the host plant," and considered that the latter was "at least by far the larger shareholder in the symbiotic relationship, if it can be regarded as such. More probable seems the view that there is no symbiosis, but that the fungus is captured and utilised by the Orchid without any compensating benefit." No direct evidence in support of either of these views was offered and no experimental work was attempted on either species. In the case of *C. innata* from the Eastern Alps, Jennings found evidence that the mycorrhizal fungus was a Hymenomycete, possibly *Clitocybe unfundibuliformis*, sporophores of which were constantly associated with the plants, but fruit bodies of *Tricholoma* sp., *Cortinarius* sp. and of a subterranean Hymenomycete were also found in close proximity.

Before leaving the subject of *Corallorhiza*, mention may be made of the observations of Lundström (1889) on *Calypso borealis* in Sweden. This rare Orchid possesses a "coralloid" rhizome with a local development of endotrophic mycorrhiza resembling that of *Corallorhiza*. The observation recorded by Lundström that mycorrhiza is not always formed in *Calypso* was confirmed later by MacDougal (1899*a, b*), who observed great variability in plants of this species, not only in respect to the formation of mycorrhiza and "coralloid" branching of the rhizome, but also in leaf and flower characters. It was noted also by Lundström that ripe fruits were difficult to find and that

seedlings were rarely seen in Nature. Moreover, attempts to raise plants from seed were not successful.

The possession of endotrophic mycorrhiza by numerous species, other than those belonging to the four families, Orchidaceae, Ericaceae, Epacridaceae, and Empetraceae, originally cited by Frank, had been established by the researches of Schlicht and Johow. Janse (1896) extended the already lengthy list by recording the wide distribution of this type of root infection among tropical plants. His attention was attracted to the subject when working on the fungus parasites of Coffee in Java, and his observations were made on plants from the forest of Tjibodas and the Botanic Garden and its neighbourhood at Buitenzorg. His researches extended over a wide field, including Bryophytes, vascular Cryptogams, Gymnosperms, Monocotyledons, and a large number of woody Dicotyledons. Of the 75 species studied, 69 showed typical endophytic infection without damage to the cells of the host, affecting the roots or rhizomes according to the habit of the species.

Certain special organs mentioned by earlier workers were studied in greater detail by Janse. His observations on this matter have a bearing on those of subsequent workers and may be briefly summarised as follows.

Soon after infection the endophytic mycelium formed terminal swellings—"vésicules"—varying in shape and size from spherical bodies 20μ in diameter to elongated sacs $100\mu \times 27\mu$ or larger. They contained much granular cytoplasm, and when mature, became gorged with reserve products, especially oil, and often acquired thicker walls of a brown colour. They were formed both within cells and in intracellular spaces and were regarded as analogous with similar structures recorded by Bruchmann (1874) and Goebel (1887) for *Lycopodium* sp., Kühn (1889) in *Angiopteris*, Schlicht (1889) in *Paris* and *Ranunculus*, Groom (1895) in *Thismia aseroë* and Poulson (1886) in *Sciaphila* sp. In common with a majority of other observers Janse was inclined to regard them as of the nature of resting spores which functioned as a means of asexual propagation for the endophyte. In general, "vésicules" were absent from Orchid mycorrhiza, but in the case of two genera, *Platanthera* and *Epipactis*, Mollberg (1884) had recorded structures believed by Janse to be analogous to them.

In describing organs of another type formed by the endophytes, Janse was on less sure ground, and it is clear from his account and from the figures illustrating it that he found it difficult to account

satisfactorily either for the structure or functions of these so-called "sporangioles." They were ubiquitous in distribution but were absent from the mycorrhiza of Orchids, where they appeared to be replaced by other structures, and also from that of seven tropical species other than Orchids. They were always intracellular, in distribution; they were formed in one or more layers of cells in the more deeply situated cortical tissues; they were rounded or irregular in shape and showed a mammillated structure interpreted as due to the inclusion of "sphérules" filled with granular material (Pl. I). Janse was evidently disposed to regard the "sporangioles" of his tropical species as analogous to the "corps jaunes" of Wahrlich in Orchids, the "dichte Massen" of Schlicht in other species, and also to the structures "which break up into bacteriods," described by Groom in *Thismia*. In view of the elucidation of their real nature by subsequent workers, it is unnecessary at this point to discuss them further.

Although Janse's paper was, in the main, an account of the morphology and anatomy of mycorrhiza in tropical plants, he did not ignore its physiological significance, and frankly admitted the necessity for isolation and study of the endophytic fungi. He referred to the unsatisfactory character of the attempts made by previous workers, and to his own unsuccessful efforts in the case of the endophyte of Coffee.

His speculations on the biology of the relationship in mycorrhiza were obviously affected by the fascinating and successful researches of Winogradski (1895) on *Clostridium pasteurianum* and by the work of Beyerinck (1888, 1890) and Laurent (1889) on the nodules of leguminous plants. Pointing out that the assistance rendered to the vascular plant by the fungus might take the form of inorganic salts, of organic substances other than nitrogenous, or of nitrogenous material, he marshalled the indirect evidence for the fixation of atmospheric nitrogen by the endophytes with the subsequent transfer of nitrogenous material to the host plant, and concluded a somewhat unconvincing argument for a reaction towards oxygen, resembling that in *Clostridium*, as follows:

L'endophyte étudié est un champignon aérobie facultatif de même que le *Rhizobium* et le *Frankia*. Il habite la grande majorité des plantes les plus diverses, et se loge dans les couches internes de la racine où il vit aux dépens des hydrates de carbone de son hôte. En pénétrant dans les tissus vivants, il cherche surtout à éviter l'oxygène. Dans ces conditions il a la faculté de fixer l'azote atmosphérique. La plante hospitalière s'empare de la plus grande partie des matières azotée que prépare le champignon et se fait payer ainsi la nourriture hydrocarbonée et la protection qu'elle lui accorde.

It may be mentioned that the experimental results furnished by cultures of Coffee in sterilised soils in no way supported these opinions. On the contrary, the experimental plants showed equally vigorous growth in treated and untreated soils.

The interest excited by the character of the nutrition in non-chlorophyllous species and their green allies suspected of a saprophytic habit was evidenced in a series of papers by MacDougal (1898-1899). The author aimed at extending the knowledge of mycorrhiza—its occurrence, the physiological relation between the symbionts, and the effect of mycorrhizal adaptations upon development with reference to survival value in the species. Two aspects of the subject engaged his attention—the recording of fresh observations upon a number of species, and the expression of his own views respecting the saprophytic habit in general. Among the plants examined were *Pterospora andromedea*, a holosaprophytic member of the Monotropeae, eight Orchidaceous species recorded as *hemisaprophytes*, including *Calypto borealis*, to which reference has already been made (see p. 41), together with *Podophyllum peltatum*, *Sarracenia purpurea* and other autotrophic species found to be free from mycorrhiza. Of new observations may be noted: the presence of stomata in species of *Epipogon*, *Aphyllorchis*, *Lecanorchis*, *Cotylanthra* and *Pterospora*, from which as from other non-green saprophytes their absence had been recorded by earlier observers, and the penetration of the endophyte into tissues other than those of absorbing organs. It does not appear that the cytology was studied in detail, but in *Peramium* (*Goodyera*) *repens*, intracellular mycelial masses were described as structures of an "absorptive character," not in any sense homologous with or analogous to the "sporangioles" of non-orchidaceous species as indicated by Janse. Despite which fact, the author appears to have regarded both "sporangioles and hyphal clumps" in general as "organs of nutrition." Although not figured, there can be little doubt of the real nature of these intracellular structures described by MacDougal; in common with "sporangioles," they will be considered more fully in a later paragraph.

The opinions expressed by MacDougal respecting nutrition of saprophytic species were founded on anatomical considerations and must be regarded as entirely theoretical. Emphasis was laid on the fact that the young seedlings of ordinary autotrophic plants are saprophytic in nutrition. The existence of this habit in the adult can be explained therefore by assuming retention of a juvenile character together with loss of chlorophyll and the development of certain structural modifications of the absorbing and transpiring organs.

MacDougal applied the term *holosaprophyte* to all species lacking chlorophyll, and *hemisaprophyte* to those showing only slight modification in structure. Accepting this customary definition, he pointed out that only three methods existed by which the requisite organic materials could be obtained by such plants:

(a) By adoption of the carnivorous habit. Since no carnivorous plant was known in which this habit was obligate, it followed that no species has attained complete saprophytism in this way.

(b) Directly, by modification of the absorbing organs, to permit the intake of organic compounds. Complete saprophytism of this kind was known only in fungi, bacteria, and—assuming the correctness of Johow's observations—in one vascular plant, the Orchid, *Wulfschlaegelia aphylla*.

(c) Indirectly, through the intervention of fungi which had invaded the absorbing organs. In this case it was assumed that "the walls of the fungus have developed a capacity for the osmotic passage of organic material....With regard to the higher plant, therefore, I have temporarily termed this adaptation *symbiotic saprophytism*," such condition being regarded as "the natural result of the supplemental capacities of two organisms brought into nutritive contact chemotropically."

MacDougal found no support for the theory advanced by Janse that root fungi were negatively chemotropic to oxygen, and under anaerobic conditions bore a relation to the host plant similar to that shown by the nodule bacteria in legumes. Moreover, he distinguished two types of endotrophic mycorrhiza, "one adapted for nitrogen fixation, and a second for the absorption and modification—perhaps oxidation—of the soil products before liberation in the tissues of the higher plant." Unfortunately, in spite of the interest of his observations, MacDougal was not able to support his views on nutrition by reference to experiment, or to contribute any new facts respecting the identity of the fungi concerned in the formation of mycorrhiza.

Evidence of a growing interest in cytological problems was provided by a new contribution to the already extensive literature on *Neottia*. In the preface of this paper Magnus (1900) referred to the dearth of observations on plant material relating to cell pathology, the contributions of Vuillemin (1890) on mycorrhiza, and of Tubeuf (1895) on diseases of plants containing little information as to changes in nuclear structure or in the finer cytological details.

It was with the view of filling this gap that Magnus undertook a comparative study of the cytology of the infected cells of Orchid roots with special reference to *Neottia nidus avis*. His observations

correlated and explained those of earlier observers in respect to certain features; the paper recording them forms a starting point for modern work on the subject, and the facts described in it have since become a commonplace of the literature dealing with Orchid mycorrhiza.

A correct interpretation of the slimy brownish masses present in many of the root cells of *Neottia* and other Orchids, due in the first instance to Cavara (1896), had been confirmed by Chodat and Lendner (1898), who, in an account of the mycorrhiza of *Listera cordata*, had described the degenerative changes of mycelium and other cell contents which took place in the older tissues. Dangeard and Armand (1898) had made a similar contribution in respect to *Ophrys aranifera*. These views had replaced the incorrect and often confused interpretations offered by Drude, Reinke, Mollberg and Wahrlich.

In *Neottia*, Magnus recorded invariable infection of the vegetative organs involving three or four layers of cortical tissue in the roots, and sometimes as many as six layers of sub-epidermal cells in the stems. Two types of infected cell were described, differing markedly in structure and physiological reactions and unconnected by transition forms—"Pilzwirthezellen" in which the mycelium retained its identity and persisted in the active condition, and "Verdauungszellen" in which it underwent rapid degeneration.

In both types of cell the hyphae on entering became clearly associated with the cell nucleus and branched extensively. In both, subsequent to infection, the mycelium was completely invested by the cytoplasm of the host cell, the latter showing a similar degree of vitality in infected and uninfected cells. In the "Pilzwirthezellen," the cell cavity became filled with mycelium showing a differentiation to thick-walled hyphae of large diameter at the periphery surrounding a central region filled with fine hyphae, the whole enveloped by the cytoplasm of the host cell. The "Verdauungszellen" in the earlier stages were filled with closely wound skeins of thin-walled protein-rich hyphae—the "Eiweisshyphen." Later, the nuclei of these cells underwent remarkable changes of size, shape and chromatin content, indicating marked functional activity, and the mycelial contents, surrounded by and including a proportion of the cell cytoplasm, became "clumped" around them, the whole contents of each cell eventually forming an opaque structureless mass—the "gelblichen Stoffes" of Schleiden and "corps jaunes" of Wahrlich and subsequent observers.

"Clump" formation involved the whole or a part of the cell

contents and was followed by complete degeneration and digestion of the mycelium. As described by Magnus, each "clump" became surrounded by a layer of cellulose-like material secreted by the cytoplasm. Whether this actually was the case or whether the substance of the clumps—apart from the cytoplasm—consisted entirely of alteration products of the digestion process, remained somewhat uncertain.

Certain features of interest emerged from this first clear and accurate account of intracellular digestion in Orchids. The two types of cell in *Neottia* were described as showing a regular arrangement, the "Pilzwirthezellen" occupying the middle part, and the "Verdauungszellen" the outer and inner layers of the infected region. Magnus reported a similar differentiation of the mycorrhiza cells in other Orchids, varying in degree in different species, and proposed a classification of Orchid mycorrhiza on this basis.

No transitions were observed between the two types of cell. In the one case, the hyphae retained their activity throughout the life of the root, in the other they invariably suffered complete degeneration. In the former, the fungus grew as a parasite, caused injury to the cell, and eventually formed resistant hyphae especially adapted to survive the winter after the death of the roots; in the latter, the products of digestion were at the disposal of the host plant. Physiologically the two types of cells were regarded as representing profit, in the one case exclusively to the fungus, in the other exclusively to the vascular plant.

Much attention was devoted by the author to the observation and interpretation of the nuclear changes observable in infected cells—their increase in chromatin content and stainability, and, in the case of "Verdauungszellen," the amoeboid changes of shape. He does not appear to have related these appearances directly to the digestive activities of the cells and was obviously puzzled by the close association of the "parasitic" hyphae and their "haustorial" branches with the cell nucleus, in relation to current views on the significance of the latter as the centre of nutritive activity in the cell. "Dass sich parasitäre Pilze, mit ihren Haustorien oft an den Zellkern legen und in seiner Nähe eigenthümlich verzweigen, gestattet Keinen Rückschluss auf die Bedeutung des Kernes als Nahrungscentrum der Zelle."

His observations confirmed the prevailing opinion that the Orchid endophytes, with their scantily developed soil connections, did not play an important part in the absorption of nutritive material from

the soil, finding the food supplies necessary for growth within the host cells.

Magnus compared the biological relation in the mycorrhiza of Orchids with that in leguminous nodules, regarding the "Verdauungszellen" as a mechanism whereby the higher plant benefited and the "Pilzwirhzellen" as one whereby certain vegetative parts of the endophytes, having escaped digestion, were returned to the soil as resting structures after the death of the roots.

Chodat and Lendner (1898) had rejected the view of a symbiotic relation beneficial to the Orchid plant in the case of *Listera cordata*, and regarded the endophyte as a relatively harmless parasite.

Regarding the condition in each mycorrhiza cell of *Neottia* as a life and death struggle for the individual symbionts, Magnus envisaged the possible existence of other and extreme types of symbiosis in other Orchids—destruction of the invaded cells at one end of the series, complete digestion of the invading mycelium at the other. A discussion on the biological significance of mycorrhiza in non-chlorophyllous plants concluded with a warning against accepting premature conclusions regarding the biological relations in view of the ignorance that existed at the time in respect to the general relations of these plants with their environment.

He also alluded briefly to the mycorrhiza of *Andromeda polifolia*, which had been figured by Frank without reference to the condition of the intracellular mycelium, and to his own observations on *Erica* and *Vaccinium* which suggested a differentiation into "Pilzwirhzellen" and "Verdauungszellen" in these genera of Ericaceae. It was also pointed out that a figure of the root cells in *Calluna* published by Pfeffer (1897) showed cells of the former type only.

Magnus contributed nothing fresh to knowledge of the identity of the root fungi of *Neottia* or other Orchids.

Lendner (1895) had re-examined the endophytes of *Platanthera* and *Vanda* from this point of view, and had accepted Wahrlich's conclusions with regard to their systematic position. Chodat and Lendner (1898) also reported that the endophytic fungus of *Listera cordata* resembled the *Nectria* isolated by Wahrlich from species of *Vanda*.

The observations of Shibata (1902) on certain Japanese plants form an interesting corollary to the work of Magnus on Orchids. Cytological investigation of two species of *Podocarpus* and of *Psilotum triquetrum* showed that the richly developed intracellular mycelium in the cells of the roots and rhizomes respectively under-

went rapid digestion, with subsequent disappearance of the products. At the onset of digestion, the nuclei of infected cells exhibited features indicating great metabolic activity—increase of size and chromatin content, amoeboid changes of shape and multiplication by amitotic division, the latter being regarded by the author, not as a symptom of degeneration, but as a means of rapid increase of the centres of functional activity in each cell. After digestion, the nuclei and host cells reverted to their ordinary condition.

The mycorrhiza of *Podocarpus* was developed as serial rows of root tubercles formed by increase in number and modification of the lateral roots; subsequent to digestion the tubercles disintegrated and were not liable to reinfection. According to Shibata, the material remaining in the cells after digestion and absorption consisted of the remains of the hyphal membranes, surrounded by an amyloid-like material. The "vésicules" formed by the endophyte were regarded as comparable with the swellings on vegetative hyphae that appear under certain cultural conditions. (Pl. I.)

The presence of active proteolytic enzymes in the tubercles of *Podocarpus* was determined by extraction of the tissues in glycerine and estimation of the changes induced in fibrin by the extract. Shibata considered that the endophyte absorbed some part of the necessary carbohydrate food material from the host plant, the symbiotic relation consisting in the acquisition of certain protein substances by the latter. In view of the scanty connections with the mycelium outside the roots, he regarded it as doubtful that the plant could benefit by reason of absorption of mineral salts by the fungus, and he preserved an open mind with regard to the general method of nutrition of the latter.

In *Psilotum* the fact of infection had been recorded previously by Solms Laubach (1884), Janse (1896), and Bernatsky (1899). In this plant it was the rhizome which suffered invasion, and the infected cells showed differentiation to "Pilzwirhzellen" and "Verdauungszellen" as in Orchids.

Shibata's observations on *Psilotum*, as well as those on the root tubercles of *Alnus* and *Myrica*, are dealt with in later sections of the present work.

Petri (1903) subsequently repeated Shibata's observations on species of *Podocarpus* with special reference to the rôle of the organs described as "sporangioles." Like the latter observer, he was able to prepare from the roots a glycerine extract with marked proteolytic properties.

EXPLANATION OF PLATE I

- Fig. 5. *a*, *Dictyostegia orobanchioides*; *b*, ditto, leaf-scale from rhizome; *c*, *Pogoniopsis* sp.; *d*, *e*, *f*, *g*, *h*, *Sciaphila schwackeana*.
Fig. 6. *a*, *Voyria tenella*; *b*, ditto, seedling from dead wood; *c*, *Voyria uniflora*; *d*, *Voyria trinitatis*.
Fig. 7. *Pogoniopsis* sp.; tr. section of lateral root.
Fig. 8. *Apteria setacea*.
Fig. 9. *Burmannia capitata*; plant growing in dead wood.
Fig. 10. *a*, *Voyria obconica*; *b*, *Burmannia capitata*; *c*, *Gymnostegia refracta*; *d*, *Dictyostegia orobanchioides*.

(From Johow, *Prings. Jahrb.* vols. 16 and 20.)

(To be continued.)

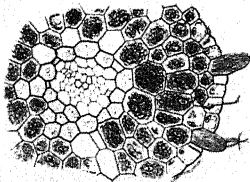
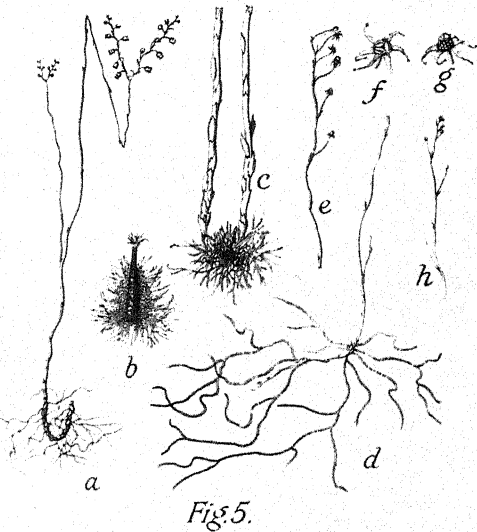


Fig. 7.

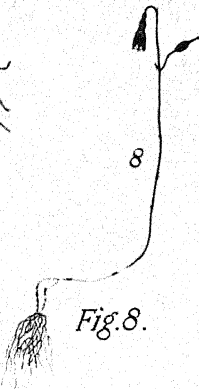


Fig. 8.



Fig. 9.

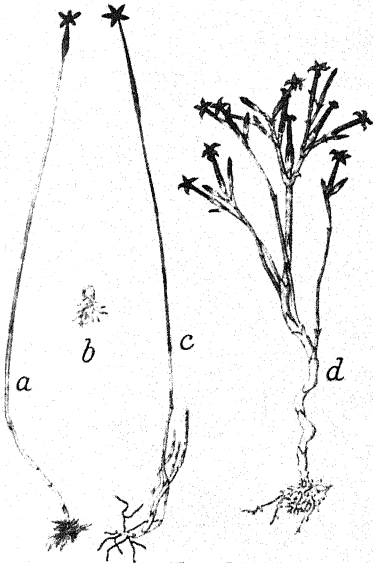


Fig. 6.

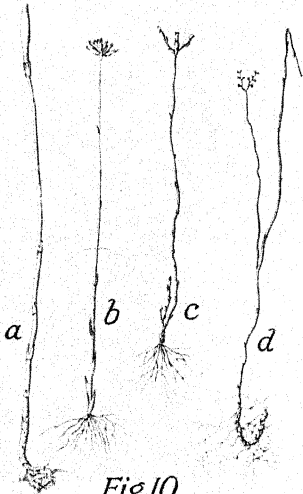
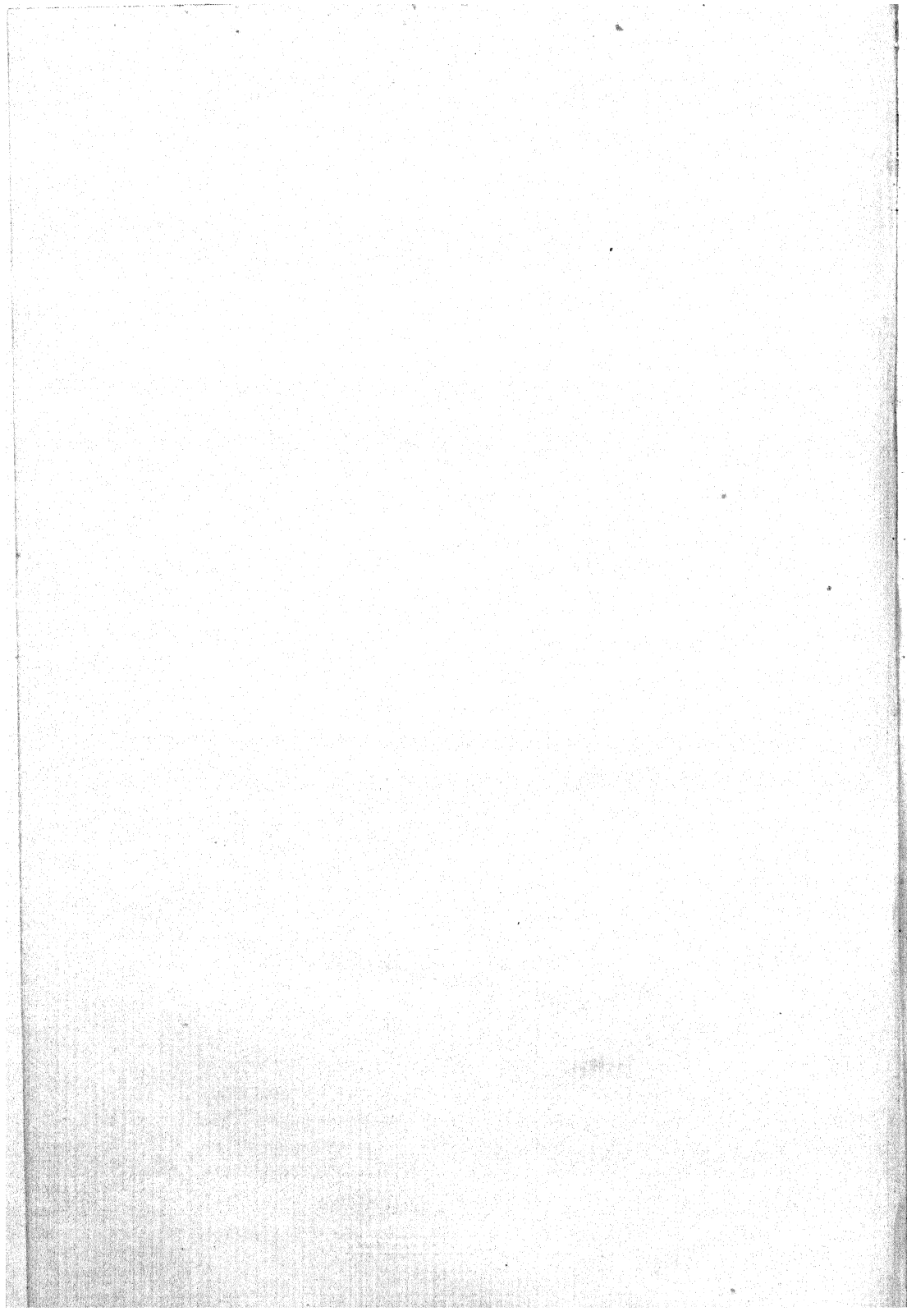


Fig. 10.



SOME OBSERVATIONS ON
BATRACHOSPERMUM MONILIFORME

By KATHLEEN M. GOODWIN, B.Sc.

THE general colour of *Batrachospermum moniliforme* in common with that of many of the lower forms of algae seems to be subject to quite a wide range of variation, and in this paper a short description is given of some experiments made for the purpose of studying the conditions under which the development of phycoerythrin or the loss of chlorophyll leads to a predominance of the red colour.

For use in certain class work on the Rhodophyceae *B. moniliforme* of a distinctly mauve colour was obtained from a stream at Sevenoaks, while in April of the following year I obtained material of an olive-green colour from the same source and further material from Merstham. I was informed by Prof. R. R. Gates that in some lakes in the north of Italy, *Batrachospermum* of a bright purple colour occurs. Specimens which I obtained from a single locality were found to differ very much in the character of their pigmentation and it appeared to me quite probable that a change in the general colour of the alga might be brought about under experimental conditions in the laboratory, and some idea formed of the factors which under natural conditions lead to the predominance of that particular pigment to which the colour of the alga is due, and also of the possible cause for the temporary change from one colour to another.

Considering the probable factors bearing on the production of the red pigment it occurred to me that the colour of the alga might normally be a matter of seasonal change, the olive-green colour occurring in early spring while the red pigmentation develops at a later period as the stream becomes more and more shaded by the vigorously growing vegetation. This idea was to a certain extent supported by the fact that olive-green material had been collected in April, before the vegetation of the streams had become very thick, while other material of a purple colour had been obtained in December. To some extent also the temperature of the water might be responsible for the control of the colour development. The Italian forms had been growing on the shaded side of the lake where the temperature (for the lake was in the mountains) was considerably lower than that of the water of the stream at Sevenoaks. It also

occurred to me that the iron content of the water might influence the development of the red pigment. A comparison of the water of the stream with that of the water used in the experiments described below showed that the latter contained a higher percentage of iron than the former.

Owing to the short time at my disposal for this work I decided to carry out at first some simple experiments for the purpose of determining what difficulties would arise in the attempt to keep *Batrachospermum moniliforme* alive in the laboratory, and, if possible, to try the effect of keeping some of the olive-green material under conditions of shade approximating to those which would occur in the stream from which the material was collected. It was found after a few experiments that the material would remain healthy and vigorous if kept in running water and plentifully supplied with air. A simple means for doing this was readily devised to suit the available facilities.

A supply of *B. moniliforme* of the usual olive-green colour was found at Merstham, and some of this was collected still attached to stones from the bed of the stream. The stream is a fairly fast-flowing one and the soil of the neighbourhood mainly chalk. In order to furnish the necessary conditions for the experiment a sink was chosen which, from its position in the laboratory, allowed some of the material to be exposed to the full available light, and some to be shaded so as to receive very little light. The material to be fully exposed was placed in a rectangular glass jar, about four feet vertically below the tap, and by means of a long piece of glass tubing into which the tap was allowed to run the material was supplied with both air and water. The rate of flow was so adjusted that the water in the rectangular jar was completely changed in one hour. By means of a siphon tube of glass the overflow from this jar was made to pass into a flat dish placed in a corner of the sink and so shaded that the material in it received very much less light than that in the jar, as nearly as possible corresponding to shade conditions, while the rate of flow and the aeration were practically the same. Neither of the specimens was exposed to direct sunlight. The experiments were commenced in April; the temperature of each bath was taken every morning at 9.30 and found to vary very little from 12° C. The water was slightly alkaline, having pH 7.5. Portions of the material were examined from time to time to see if any changes occurred. Under these conditions the alga remained perfectly healthy during the period of six weeks over which the experiment

extended. Some of the green material was placed in a dish in the sink occupying a more exposed position in the laboratory and supplied with air and water as before. This green material had cystocarps at the time the experiment was set up but at no time were there any seen on the olive-green material.

After the material had been kept under these conditions for three weeks it was found that the stone in the darkest corner of the shaded dish was covered with a red jelly-like mass, while another colony in the same dish had patches of red material amongst the olive-green. The unshaded material in the rectangular jar had undergone no apparent change. On examining the material microscopically it was seen that the filaments were perfectly normal and the growth quite healthy. The red pigment appeared quite typical of that seen in the Rhodophyceae and continued to ooze out from the filament when any material to be examined was placed in distilled water. It has not been possible so far to extract and examine the pigment itself, but there seems little doubt as to its being similar to the phycoerythrin examined and described by Hansen. As it seems doubtful, however, whether the chemical and physical properties of a carefully made pure preparation would materially assist in the interpretation of the behaviour of the pigment in living material, an attempt is being made to devise some means for the study of this in the living condition.

The red colour of the group Rhodophyceae is primarily due to phycoerythrin, a pigment of unknown constitution, masking the normal chlorophyll present. This red pigment usually occurs in these algae in deep water although fresh water forms may also be red or purple. The physiological function of phycoerythrin has been described as complementary to that of chlorophyll. It has been shown by Hansen that phycoerythrin absorbs that part of the spectrum penetrating deep water, and that only light of 600-486 excites fluorescence in phycoerythrin solutions. He was able to photograph fluorescent light from phycoerythrin solutions and found two well-marked bands near the *D* line and at the hydrogen end (656-680 and 600-570). These seemed related to the absorption bands of chlorophyll. Blue-green light produced the same fluorescence as white light, hence he states that blue-green light causes an orange fluorescence with bands in the same position as chlorophyll absorption bands I and III, so that phycoerythrin assists assimilation by absorbing blue-green light and degrading it to those wave lengths absorbed by chlorophyll.

The fact that colour variation occurs in a single species points to the possible formation of a series of compounds of which the red may be the final form, green developing into olive-green, then through olive-brown to red. Again, the olive-green coloration might possibly be due to a degradation product of phycoerythrin. It also seems quite probable that under these conditions an amount of carotin above the normal might be formed since it is reported as a normal constituent of most members of the Rhodophyceae by Reinke, Tammes and others.

SUMMARY.

1. *Batrachospermum moniliforme* may be grown in the laboratory without much difficulty, if supplied with running water and well aerated. A simple method of doing this is described. The water used in these experiments had a high iron content and a slightly alkaline reaction, pH 7.5.

2. The form having an olive-green colour when collected, if grown in diffuse light, corresponding to natural shade conditions, gradually loses its olive-green colour and becomes red.

3. The red material obtained in this way is quite normal in appearance and as far as could be judged it was perfectly healthy at the close of the experiments.

4. The development of the red colour under the conditions of the experiments is in keeping with the function ascribed to phycoerythrin by Hansen.

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NOTE ON A NEW FORM OF ELECTRICALLY DRIVEN KLINOSTAT

By EDGAR RHODES

(Demonstrator in Plant Physiology, University of Leeds.)

IN this department, the need arose for a robust and reliable form of Klinostat which could be relied upon to run for long periods with the minimum of attention. The ordinary type actuated by clockwork, having in our experience proved extremely unreliable and as there appeared to be no better form on the market, an electrically driven type was devised, and this having proved very satisfactory it was thought to be worth while bringing it before the notice of any who may need such an apparatus in a compact and useful form. In general arrangement, it is somewhat similar to that described by Sperlich (*loc. cit.* p. 511) from which paper some valuable hints were obtained.

The photograph (Text-fig. 1) shows the complete apparatus consisting of a $\frac{3}{8}$ in. thick metal bed measuring 30 in. \times 12 in., upon which are mounted rigidly a $\frac{1}{4}$ th H.P. motor with sleeve coupling¹ to a reduction gear box, and the supports which are 18 in. high and which carry the Klinostat shaft. This shaft is of $\frac{1}{2}$ in. mild steel turning in ball bearings mounted in the supports, and carries a bicycle gear wheel which takes the drive from a small sprocket wheel on the gear box shaft. The motor is driven from the only electric mains available, which are 110 volts A.C., and it was obviously an advantage for it to develop its power at the lowest possible speed. The slowest motor available was one giving nearly 1500 revolutions per minute, so that the problem of gearing was to effect a reduction from this speed to $\frac{1}{20}$ th revolution per minute, and although such a reduction (30,000 times) is unusual, it has been very satisfactorily and fairly simply achieved. It might also be mentioned that in order to obviate the use of a starter, the motor fitted incorporates a special starting winding allowing speed to reach maximum only relatively slowly, and cutting out automatically when the maximum is reached. By this means the starting mechanism is reduced to a simple switch and undue snatch on the gears is avoided.

¹ Since its inception, a further improvement has been made by substituting a flexible coupling for the sleeve coupling illustrated in Text-fig. 1.

The gear box is built on substantial lines, as is very necessary if it is to effect such a large reduction and at the same time be capable of standing up to continuous use over long periods. It has been

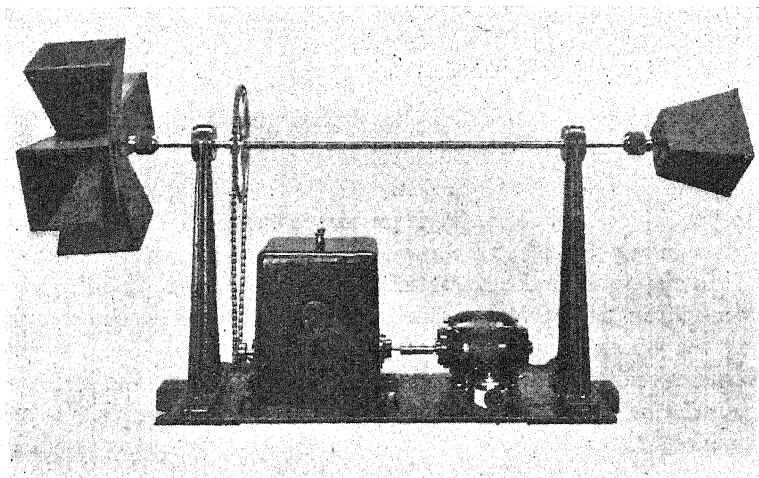


Fig. 1

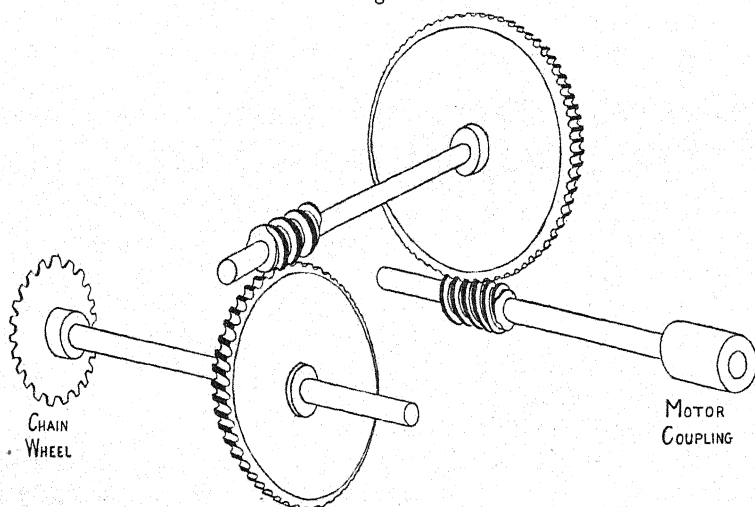


Fig. 2

built with an eye to prevention of wear especially in the more rapidly moving parts. Text-fig. 2 shows the principle of the gearing, which is a series of worm reductions. The actual size and number

of teeth on the wheels being dependent upon the rate at which the motor is designed to revolve, and since it is difficult to obtain two motors with the same speed, it would serve no useful purpose to quote these engineering details. It must be added, however, that all gears are cut from phosphor bronze blanks, and the free ends of each worm and worm wheel shaft run in ball bearings mounted in small ball races fixed in the gear box casing. The gear box casing itself is of cast iron, encloses all the mechanism, and carries at the top two grease cups, which only need to be re-filled occasionally to keep the whole system well lubricated. The whole apparatus runs very freely, and apart from the hum of the motor is noiseless in action.

The arrangements for carrying plants are simple. Each end of the Klinostat shaft carries a small lathe "chuck," and wooden plant pot holders of different types have been obtained, each one having a short steel rod attached to its base so that all that is necessary in fixing is to place the rod in the jaws of the chuck and screw down firmly. The pot or pots in the holders are thus rigidly held on the rotating shaft. It will be seen that an arrangement of this kind possesses many possibilities. It is for instance an easy matter to devise a pot holder which will carry four or even eight pots attached to a central block on which is mounted the small steel attachment rod, the whole turning at one end of the shaft. If desired, as in the four pot holder illustrated, the pots can be made to turn in the plane at right angles to the shaft line. The illustration also shows a single pot holder being rotated in the normal position. There is no possibility of over-weighting the machine, for a load of several hundred-weights, placed several inches out of alignment, can be turned as easily and safely as a single pot; so that by simply obtaining suitable cheap wooden holders, a single Klinostat of this type can be made to do the work of eight or even sixteen ordinary machines. The same slowly revolving shaft provided with an additional bicycle gear wheel and chain would turn another shaft mounted like itself producing virtually a second Klinostat.

The machine illustrated was designed by Mr C. H. Richardson, of Messrs Orme and Co., Ltd., Apparatus and Instrument Makers, Russell Street, London Road, Manchester, in collaboration with Prof. J. H. Priestley and the author.

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NOTE ON A NEW METHOD FOR THE INVESTIGATION OF FOSSIL PLANTS

BY T. M. HARRIS

I N the course of work on the fossil plants collected by Dr N. Hartz from the Rhaetic beds of East Greenland and lent by him for investigation, a method was devised by which the whole of the more resistant plant remains are rendered accessible, and not merely those exposed on split surfaces of the rock. The method was later found to be applicable to a large range of rocks containing plant remains in the mummified condition.

DESCRIPTION OF THE METHOD.

1. The shale is immersed, for a few days, in strong nitric acid containing about 5 per cent. of potassium chlorate. The fragments swell owing to the evolution of gas by the oxidation of organic matter.

2. The acid is removed and the last traces washed out by running water.

3. The shale is placed in dilute sodium hydroxide. It breaks down into fine mud while part of the organic matter goes to form a black solution.

4. Resistant fragments are strained off through a series of wire gauzes.

5. The fragments are finally cleaned with 25 per cent. hydrofluoric acid. They consist of the cuticle alone or, if the acid treatment has not gone too far, of the softer tissues as well. The fragments are naturally rather small, rarely exceeding 2 cm. in length, but they can be readily distinguished by their cuticular structure with a binocular microscope giving a magnification of about 200 diameters.

EXAMPLES OF USEFUL APPLICATIONS.

1. Specimens of stratigraphical use and hitherto very rare were found by this method. Thus two of the characteristic megaspores of *Lycostrobus Scotti* Nath. were discovered, the only other specimen known having been obtained from the Rhaetic of Sweden. Several other fossils of great assistance in the determination of the horizon of the beds were discovered by this method.

2. A great number of leaf fragments were obtained (sometimes the bulk of wet cuticles exceeds that of the original shale) and some of them showed finer details than the leaves exposed on a split surface.

3. Small seeds, bud-scales, etc. are often found in great numbers associated with fossil leaves.

(a) From a piece of shale consisting mainly of *Sagenopteris Nilssoniana* (Brongn.) Ward, leaves, pollen grains, anthers and seeds were obtained which were scarcely distinguishable from those described by Dr H. Hamshaw Thomas in his paper on the Caytoniales. Neither these organs nor *Sagenopteris* leaves were found in macerations of other shales. This association, which could not have been recognised without this method, greatly strengthens the evidence for Hamshaw Thomas's view that the leaves of the Caytoniales were of the *Sagenopteris* type.

(b) From a shale in which the chief fossils are *Anomozamites minor* (Brongn.) Nath. leaves and Bennettitalean reproductive bracts, great numbers of interseminal scales were obtained resembling those of *Wielandiella angustifolia* described by Nathorst in 1909. In addition about a hundred characteristic small seeds of a new type were found which are probably referable to *Wielandiella*.

4. Sometimes evidence is furnished which throws light on the gross features of a fossil which is not preserved entire. Thus the most complete fragment of a leaf of a new species of *Phoenicopsis* was 5 cm. in length. Several kilograms of the shale in which this leaf occurs were macerated. The length of an average leaf was found from the ratio

$$\frac{\text{Total length of all the fragments}}{\text{Number of apices present}}$$

to be 15 cm.

This would have been impossible without using the bulk-maceration method; for the figures obtained would be too small to give a significant result, and any conclusion would be open to objection if it had not been known that the whole of every leaf in the shale had been examined.

Many small pieces of charcoal were found. They were softened with Eau de Javelle, and about one-third of them could be teased into radial sections showing the pitting of the tracheids and medullary rays. Seventeen types were obtained, of which eleven were of the Araucarian type, five Abietineous, and only one Pteridophyte. As about fifty fragments were examined they provide some idea of the

type of wood prevalent at the time. Specimens of petrified wood are so seldom met with in Mesozoic rocks that the few specimens investigated from any one locality furnish little evidence on this point. Only once was any charcoal exposed on the split surface of a rock specimen; all the other charcoal specimens were obtained by the bulk-maceration method.

5. A number of small bodies, possibly eggs, were obtained.

The method was of use in one way or another for more than half of the total number of species (106) at present known from East Greenland. It has therefore led to the recognition in this collection of one of the largest early Mesozoic floras known.

I have been able to obtain well-preserved leaf remains by this method from the Permian of Nottinghamshire and from the upper Estuarine clay of the Jurassic of Northamptonshire and of Yorkshire.

Several palaeobotanists have used similar methods for obtaining spores from shale, e.g. Nathorst (1908) who macerated a few grams of Swedish Liassic clay, but such methods do not yet seem to be recognised as useful on a large scale.

Bryophyte remains have been obtained by J. Walton (1925) by the maceration of some carboniferous shales with hydrofluoric acid. The disintegration of material in bulk by this reagent is however very inconvenient, and while its use is unavoidable with hard sandstones and shales, I have found it to possess no advantage whatever over the method here outlined for the softer rocks with which I have worked.

THE BOTANY SCHOOL,
CAMBRIDGE.

January, 1926.

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INTERNATIONAL CONGRESS OF PLANT SCIENCES. (FOURTH INTERNATIONAL BOTANICAL CONGRESS)

COMMUNICATED BY PROF. B. M. DUGGAR
(Chairman of the Organizing Committee)

INVESTIGATORS and teachers in the plant sciences, representing all aspects of botany, plant chemistry, plant pathology, and bacteriology, agronomy, horticulture, and forestry are invited to attend the International Congress of Plant Sciences to be held at Ithaca, August 16-23, 1926. This invitation is extended to all countries of the world.

This Congress is scheduled to follow the usual academic sessions abroad, also the short summer session of the various universities and colleges in the United States. It is believed to offer the first opportunity ever arranged in the United States for a general conference of all those professionally engaged in plant work. It possesses the additional compelling interest accruing from unrestricted international representation.

In order that a part of the program may be representative of outstanding leadership, the Congress will be divided into about one dozen sections, each section with an invitation program occupying about four morning sessions or a little more than one-fourth of the available time. These formal programs will be supplemented by another feature that promises also to be of exceptional interest. Ample time will be set apart for round table or informal discussions, which in some cases may be scheduled in advance, and in others may be arranged both as to topics and participation after the Congress actually convenes. This is intended to provide for the widest participation in sectional activities. Supplementary opportunities for individual contact and participation are made possible through the non-commercial exhibits and through the provision for excursions and inspection tours of various types suited to the diverse needs of the different sections.

Although the Congress is not to provide an occasion for *legislation* on regulatory matters of international significance (such as nomenclatorial rules) the organizing committee has expressly provided that "adequate opportunity shall be accorded all sections for the *discussion*

of regulatory recommendations of international significance," in order that a better understanding may be reached for definite action at a subsequent international congress.

The sections thus far authorised and the secretaries representing these groups are as follows:—

Agronomy: R. G. WIGGANS, Cornell University, Ithaca, N.Y.

Bacteriology: J. M. SHERMAN, Cornell University, Ithaca, N.Y.

Cytology: L. W. SHARP, Cornell University, Ithaca, N.Y.

Morphology, Histology and Paleobotany: D. S. JOHNSON, Johns Hopkins University, Baltimore, Md.

Ecology: H. L. SHANTZ, Bureau of Plant Industry, Washington, D.C.

Horticulture: A. J. HEINICKE, Cornell University, Ithaca, N.Y.

Physiology: O. F. CURTIS, Cornell University, Ithaca, N.Y.

Pathology: DONALD REDDICK, Cornell University, Ithaca, N.Y.

Pharmacognosy and Pharmaceutical Botany: H. W. YOUNGKEN, Massachusetts College of Pharmacy, Boston.

Taxonomy: K. M. WIEGAND, Cornell University, Ithaca, N.Y.

Forestry: R. S. HOSMER, Cornell University, Ithaca, N.Y.

Genetics: C. E. ALLEN, University of Wisconsin, Madison, Wis.

Mycology: H. M. FITZPATRICK, Cornell University, Ithaca, N.Y.

Communications regarding the Congress should be addressed as indicated below:—

(1) Concerning round tables and other strictly sectional matters, to the appropriate sectional secretary.

(2) Concerning exhibits and general program matters, L. W. Sharp, Cornell University, Ithaca, N.Y.

(3) Concerning excursions, collecting trips, inspection tours, local arrangements, transportation, etc., H. H. Whetzel, Cornell University, Ithaca, N.Y.

(4) Concerning the Congress in general, B. M. Duggar, Missouri Botanical Garden, St Louis, Mo.

REVIEWS

Imperial Botanical Conference, London, 1924. Report of Proceedings,
 edited by F. T. BROOKS. Cambridge University Press, 1925.
 Price 15s.

We should like to call the attention of those of our readers who do not know it to this volume, which appeared about a year ago. The circumstances under which the first Imperial Botanical Conference came to be summoned are fully and lucidly explained by Sir David Prain, the President of the Conference, in the Foreword to this book and in the Address which he delivered at the opening of the Conference; they need not be touched upon here. The outstanding fact is that the Conference came into being and was, we think universally, considered by those who took part in it a very great success. While the botanists of the British Empire, like those of the rest of the world, are, as men of science, primarily devoted to the study of plant life as such without reference to national or imperial boundaries or sentiments, there can be no question that it is useful sometimes to focus their interest on the problems which concern them especially as belonging to the British Empire. Many of these are of course specific problems of the economic application of botany, but there are others of at least equal importance.

Among these is the subject of education in botany. To acquire broad and sound views as to what the main lines of botanical education should be it is necessary that there should be constant fertilising interaction between Dominion and Colonial and Home experience of the results of existing methods of training, their merits and their deficiencies. This subject, as such, was not discussed at the Conference, but interesting evidence of the divergent views of different botanists may be gleaned here and there from their printed remarks. Another topic falling within this general field is the strong conviction expressed by several botanists, both in the Dominions and at home, of the very great advantages that would accrue from the free interchange of teachers for periods of six months or a year between different parts of the Empire. A special sub-committee of the Executive Committee of the Conference has been quite recently considering this question and collecting evidence upon it. They have arrived at the conclusion that, beneficial as such interchange would be, practical difficulties forbid the organisation of any general scheme for carrying it out. They have however made a number of practical recommendations with the object of facilitating visits of some duration by home botanists to other parts of the Empire and by Dominion botanists to Great Britain.

Another general problem, the stimulus to attacking which is largely, but by no means wholly, economic, is the desirability of developing an organised survey of the vegetational resources of the Empire. On one side this is concerned with the more rapid and efficient preparation of handbooks to regional and local floras, and with the complicated technique involved in floristic work. The Conference provided a very useful ventilation of these matters, which especially concern our great herbaria. On another side it is concerned with the promotion of the survey and study of the types of natural and semi-natural vegetation existing within the Empire. This subject was dealt with rather fully at the Conference, which set up a permanent committee—the British Empire Vegetation Committee—to take practical steps to deal with it. The first fruits of the Committee's activity will shortly appear in the form of a handbook on "Aims and Methods in the Study of Vegetation," the thorough distribution of which throughout the Empire is being arranged for.

The other principal subjects dealt with by the Conference were Crop Physiology, Plant Pathology and Mycology, and the application of Genetics to the improvement of crops, all topics of primary economic importance. It is

impossible in this notice to indicate, however briefly, the wide and deep interest of the contributions to these subjects contained in the printed volume.

The 1924 Conference was of the nature of an experiment, but it was certainly a successful experiment. The feeling that it should be only the first of a series was widely expressed. We may expect that the International Botanical Conferences, interrupted by the war, will soon be again established on a quinquennial basis. It would be a very great pity if these should interfere with the organisation of Imperial Conferences, which should not be separated by longer intervals. It may also be hoped that the Dominion Governments and other authorities concerned may be able to arrange in future for a fuller representation of Dominion and Colonial botanists.

Meanwhile we may cordially commend the present volume, which is full of intrinsic interest and is also beautifully produced, to the attention of all English-speaking botanists, including those of the United States, many of whose problems are similar to ours.

A NEW REVIEW OF GENERAL BIOLOGY

The Quarterly Review of Biology, edited by RAYMOND PEARL. Vol. I. No. 1. January, 1926. Pp. 1-137. The Williams & Wilkins Company, Baltimore, U.S.A. English Agents, Baillière, Tindall & Cox, 8 Henrietta St., Covent Garden, W.C. 2. Price 21s. per annum.

We have often thought that a Review of General Biology on the lines expounded by Professor Raymond Pearl in his Foreword to the first issue of this new publication was very much wanted by all those who desire to keep in touch with the progress of biology at large, but find it impossible to do so adequately from existing publications. Such a Review, to be really successful, requires very able and skilful editing. Both the General Editor and his Advisory Board must not only have a wide and deep knowledge of all the main lines of progress, but they must have impartial minds, and keep a constant watch on their contributors to see that the articles and reviews really present a fair picture of genuine advances.

The first issue contains five articles, four of which deal with animals and one with plants. The longest, by Prof. C. R. Moore, is an interesting and extended survey of recent work on the Biology of the Mammalian Testis and Scrotum, bringing out, among many other points, the fact that the descent of the testes in most mammals is correlated with inability to carry out spermatogenesis at higher (body) temperatures in those groups. The doubt still attaching to Steinach's and other views of the origin, nature and effects of the internal testicular secretion is also emphasised. It is a question, perhaps, whether it is wise to devote one-third of the issue to a single topic. Another interesting paper is on Recent Discoveries in the Biology of Amoeba. Dr Kellerman notices Garner and Allard's important work on the Influence of the Length of Daily Light Periods on the Growth of Plants. There is a not very penetrating notice of Cuénot's recent work *L'Adaptation*. Finally 14 pages are devoted to very short notices of no less than 75 different recent biological works, and these, in spite of their brevity, often succeed in giving the reader a good notion of the nature of the book noticed.

It is too early to say whether the new Review will achieve lasting success. That depends partly on whether it is possible to continue to present reviews of biological work sufficiently varied and at the same time of first class importance, and partly on the adequacy of the presentation. We must however welcome and cordially wish success to this serious attempt to meet an undoubted want.

THE NEW PHYTOLOGIST

VOL. XXV, No. 2

25 JUNE, 1926

MYCORRHIZA

By M. C. RAYNER

CHAPTER IV

The Modern Period: 1900-1925—Sarauw—Stahl: autotrophic and mycotrophic plants; theory of nutrition—Marcuse—Mycorrhiza of Arctic and Alpine plants: Hesselman; Schröter; Wulff—Gallaud.

THE MODERN PERIOD: 1900-1925.

ON the whole, the views current at the beginning of the present century respecting ectotrophic mycorrhiza tended to attach rather less importance to Frank's opinions without abandoning the theory of a symbiotic relation. Thus, Tubeuf (1903) from 1896 onwards had observed the presence of functional root hairs in the ectotrophic mycorrhiza of trees and noted that a proportion of the roots were free from fungal infection. He had recorded also the not infrequent appearance of the endotrophic type of mycorrhiza in forest trees and was of opinion that the latter obtained the necessary salts independently, the root fungi functioning only as an indirect means of drawing upon the organic compounds of nitrogen locked up in humus. Observations and experiments on forest trees led Sarauw (1903) to conclude that the fungi were relatively harmless to the trees, although he regarded it as improbable that the latter derived any benefit from the association—"Dass die Pilze unseren Waldbaumwurzeln und den Bäumen Vorteil bringen sollten, ist bisher meines Erachtens weder durch Beobachtungen in der Natur, noch durch Versuche nachgewiesen worden."

Möller (1903, 1906) had reached similar conclusions. His observation that Pines made good growth on sandy soils deficient in humus had led him to dispute Frank's view that *Pinus sylvestris* does not come to maturity on normal soils if mycorrhiza formation is hindered owing to lack of humus and the absence of the appropriate fungus; moreover, his experiments to test the possibility of nitrogen fixation

by the root fungi of Pine and Oak had yielded negative results for both these trees.

A fresh attempt to survey the whole field and correlate the problems of nutrition presented by mycorrhiza plants with those in parasites and insectivorous plants was made by Stahl at the opening of the new century (Stahl, 1900). This paper, the most comprehensive study of mycorrhiza from the biological point of view since the publication of Frank's theory of symbiosis in trees, has been freely quoted in the text-books and is probably one of the best-known contributions to the literature of the subject. Two aspects of Stahl's work demand attention: firstly, the new hypothesis put forward by him to explain the distribution of fungus infection in vascular plants and its beneficial effect upon the hosts, and secondly, the character of the experimental evidence offered in support of his opinions.

Stahl's theory of nutrition was supported by the following arguments:

(1) Both vascular plants and fungi make heavy demands upon certain essential mineral salts in the soil; moreover, investigation shows that fungus mycelium is a very efficient mechanism for removing soluble salts from the soil. There must be, therefore, a powerful struggle for the essential mineral salts in soils, more especially in those rich in humus in which fungi are specially abundant.

(2) The higher plants best fitted for competition under these conditions will be those with extensive root systems and abundant root hairs, in which a rapid transpiration current is facilitated by structural characters favourable for transpiration, e.g. by the presence of hydathodes, etc.

(3) Vascular plants of this type growing in humus soils are usually free from fungus infection, e.g. Elder (*Sambucus nigra*), members of the Cyperaceae, and various Ferns. Obligate mycorrhiza plants, on the other hand, have commonly a sluggish transpiration current, whether due to inefficient absorption, or to an ineffective mechanism for the elimination of water from the shoot, or to various interactions between these factors. On humus soils in particular, they cannot compete with rapidly transpiring species or with fungi without the assistance of the symbiotic mycelium in their mycorrhiza. The great benefit derived in this way becomes evident when it is recalled that the development of the root fungi reaches a maximum in the autumn, i.e. at the season when transpiration is slowest.

The major part of the paper consisted of an elaboration of the thesis just outlined, namely, that the incidence of fungus infection was directly related to the difficulty of procuring mineral salts, and hence, to the efficiency or otherwise of the mechanism for their absorption, it being assumed throughout that a large intake of water and a rapid transpiration current involved, of necessity, a correspondingly great absorption of nutritive salts from the soil. As a matter of convenience Stahl distinguished two groups of plants—those with and those without mycorrhiza, the former group including *obligate mycorrhiza plants*, which depended always upon root infection for their proper nutrition, and *facultative mycorrhiza plants*, which were infected or fungus-free according to the character of the substratum. In reference to nutrition, all mycorrhiza plants were described as *mycotrophic*.

Reviewing his own observations and also those of other workers on the distribution of fungus infection, and neglecting certain contradictory facts, Stahl concluded—"dass die Mycorrhizenbildung höchst wahrscheinlich mit der erschwerten Nährsalzgewinnung in irgend einem näheren Zusammenhang steht." He stressed the intensity of the competition for essential salts in the humus soils with which many of the best known mycotrophic species were specially identified, and proceeded to test his theory of nutrition by seeking evidence that mycotrophic plants in general showed anatomical and morphological characters related to their symbiotic mode of nutrition.

The details of this investigation on mycotrophic species—the relation of transpiration to sugar or starch in the leaf cells, the distribution of hydathodes and other mechanisms for facilitating the flow of water, the observed deficiency of nitrates and other salts in the tissues—need not be considered in this review. Many observations of interest were included in the argument and may have a real significance of the kind postulated by Stahl. Nevertheless, they cannot be regarded as providing convincing evidence of the general proposition that the mycotrophic habit is a special adaptation giving to certain species compensation for the handicap imposed upon them by a sluggish transpiration current. In the light of modern work on absorption it is indeed doubtful whether the assumed direct correlation between intake of water and absorption of mineral salts exists. The evidence points to the two processes being entirely distinct, i.e. that a rapidly absorbing and rapidly transpiring plant is not necessarily specially favoured in the competition for mineral salts.

Furthermore, some of the experimental evidence offered by Stahl is open to interpretations other than those suggested. For example, a method adopted was to grow comparative cultures of various species in suitable soils and in similar soils sterilised by exposure to ether vapour for five days. The experiments were designed to illustrate the potent effect of fungus competition by removal of this factor in the soils sterilised with ether, the beneficial effects observed in seedlings being attributed to the decreased competition for salts. Whether or not the differential growth actually observed in the roots of the two sets of experimental plants was due, as believed by Stahl, to the retarding effect upon growth of a relatively higher concentration of salts due to the absence of mycelium in the sterilised soils, the bearing of this observation upon the biological function of mycorrhiza is indirect, and it in no way provided experimental proof that the presence of mycelium in the root tissues of mycotrophic plants facilitated the absorption of salts from the soil. The increased vigour observed in seedlings in the treated soils is shown in the photograph now reproduced (Fig. 11).

In Stahl's earlier experiments the treated soils were sterilised by heating to $100^{\circ}\text{C}.$; in his later work, to which the series of experiments figured belong, sterilisation was effected by exposure to the vapour of chloroform or ether. In view of modern work on soil sterilisation, it is certainly unsafe to conclude, as did Stahl at the time of the experiments, that the beneficial effect observed was due solely to decreased competition for essential salts. The effects produced by *partial sterilisation* of soil, whether by heating to $100^{\circ}\text{C}.$ or by exposure to volatile antiseptics, are complex in origin, and lead directly and indirectly to an increase in the amount of available plant food present. The stimulus to growth commonly noted results from this increased supply and would mask any effect due to removal of fungus competition (Russell, 1921).

By independent observation, Stahl confirmed Frank's conclusion that obligate mycorrhiza plants always gave a negative reaction when tested for nitrates, even when growing in the same soil with fungus-free species which reacted strongly. This fact has no essential bearing on the hypothesis of a differential rate of absorption in mycotrophic as compared with autotrophic plants, significant though it may be in relation to the nitrogen metabolism of the former group. His comparative observations on insectivorous plants—their distribution upon soils poor in nitrates and other essential salts and their freedom from mycorrhiza—and his conclusions that the

carnivorous and mycotrophic habits appeared to be mutually exclusive, are of interest in the same connection.

It was assumed by Stahl, as by earlier workers and by his contemporaries, that fungus infection took place exclusively from the soil. Modern work upon Ericaceae, with the resulting experimental demonstration of the real character of the symbiotic relationship in this group, has proved this view to be erroneous and, incidentally,

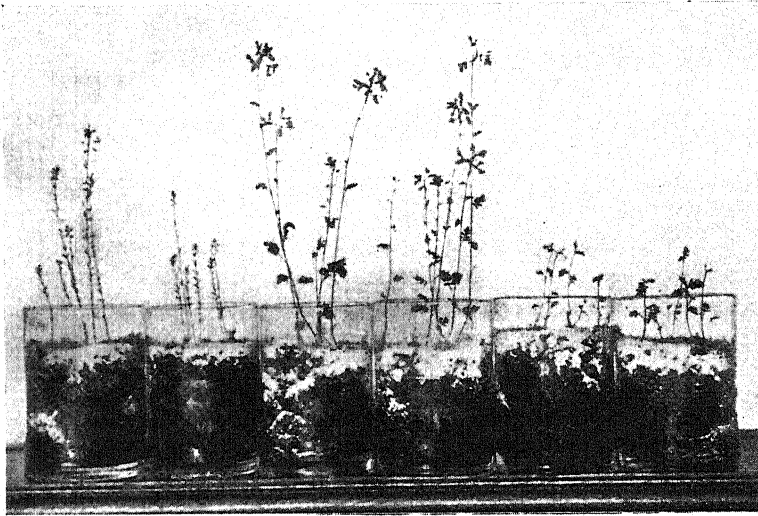


FIG. 11. Stahl's experiment to demonstrate the effect of fungus competition in soil. Left, two pots with *Linum usitatissimum*; the larger plant growing in sterilised, the smaller in unsterilised humus. Right, four pots with *Sinapis alba*; the two larger plants in sterilised, the two smaller in unsterilised humus. (From Stahl, *Jahrb. f. wiss. Bot.* 1900.)

has raised doubt as to the correctness of his observations on *Vaccinium myrtillis*. In the paper now under consideration it was recorded that seeds of this species sown on peat in May gave plants the roots of which showed beautiful and characteristic mycorrhiza when examined in the following October, whereas the roots of seedlings of the same age grown upon similar soil sterilised by heat were entirely free from fungus infection:

Hierbei stellte sich heraus, dass die Exemplare, deren Wurzeln sich in dem der Siedehitze bzw. Aetherdämpfen ausgesetzten Substrat ausgebreitet hatten, völlig pilzfrei waren. Bei den in nicht sterilisiertem Substrat wurzelnden Pflänzchen waren dagegen die für die Ericaceen charakteristischen Mycorrhizen in schönster Weise zur Ausbildung gelangt.

If, in *Vaccinium* as in *Calluna*, casual infection of the seedling from the soil is supplemented by regular infection from the seedcoat at germination, Stahl's conclusions obviously require revision. This matter will be discussed again when dealing with the Ericaceae.

Working in Stahl's laboratory, Marcuse (1902) re-investigated the mycorrhiza of a number of Orchids and other species, with a view to confirming the opinions expressed by the former respecting its biological significance. His observations on green and non-green Orchids led him to conclude that the condition and distribution of the endophytes stood in direct relation with the vegetative period, general environment of the plants, and age of the roots.

The structural evidence was held to support the view that outgrowing hyphae functioned in a similar way to root hairs; at the same time, the author reiterated the uncertainty respecting the ability of non-green plants to draw directly upon the humus constituents of soil, i.e. he questioned whether they were, in the strict sense, saprophytic in nutrition. Among the species examined, other than Orchids, were the following—*Botrychium lunaria*, *Linum catharticum*, *Polygala amara*, *P. vulgaris* and various members of Prolaceae, all of which are typically mycotrophic plants. Heinricher (1900) had previously rejected the hypothesis of a parasitic habit for the genus *Polygala*, recording *P. chamaebuxus* as an obligate mycorrhiza plant and other species of the genus as facultatively mycotrophic. The figure of a root section of *P. amara* supplied by Marcuse suggests that typical intracellular digestion occurs in the latter species, although this matter is not mentioned by the author (Fig. 17). A few new cases were added to the rapidly growing list of plants known to form endotrophic mycorrhiza, but in the main this paper may be regarded as consisting of special pleading in support of the Stahl hypothesis; namely, that the demand for available mineral salts in certain soils exceeds the supply, the resulting intensity of competition being the primary cause of a symbiotic relation in mycorrhiza.

A somewhat new aspect of mycorrhiza was opened up by Hesselman (1900), who examined a number of Arctic species from Bear Island, Spitzbergen, and other northern stations during a Swedish polar expedition in the summer of 1898. Well-developed mycorrhiza of various types was recorded for *Salix* sp., *Dryas octopetala*, *Diapensia lapponica*, *Taraxacum phymatocarpum*, *Arnica alpina*, *Erigeron uniflorus* and *E. compositus*. In *Polygonum viviparum* it was of typical ectotrophic structure, an exceptional condition in herbaceous plants.

The formation of mycorrhiza by Arctic and Alpine plants is not in itself remarkable, but its existence was so bound up with the theory of nutrition from soil humus, that, at the time of Hesselman's observations, it must have occasioned some surprise to find root infection well developed in plants growing in poor and sterile soils in which a deficiency of humus constituents was determined both by the paucity of plant remains and by the unfavourable climatic conditions for bacterial action. Hesselman described mycorrhiza in *Dryas octopetala* from stations so diverse as Spitzbergen, Nova Zemlya, North Europe, Tirol, the Apennines and the Altai Mountains; the variety *integrifolia* was said also to be typically infected.

Subsequently, Schröter (1908) figured the mycorrhiza of *Pinus montana* and various herbaceous Alpine plants including the grass *Sesleria coerulea*. In the last-named species, root infection was found to be sporadic and was recorded as possibly related to the character of the habitat. In another grass, viz. *Nardus stricta*, Schnellenberg had described mycorrhiza of the endotrophic type. In this case the incidence of infection was correlated with well-marked differentiation to long and short roots, the latter characterised by arrested growth, profuse branching, absence or poverty of root hairs, and heavy intracellular infection. Stahl (1900) had previously recorded the formation of mycorrhiza by many Alpine species of Gentian.

In connection with Stahl's hypothesis that the mycotrophic habit is correlated with a difficulty in obtaining mineral salts, with feeble transpiration, and with poverty or absence of starch, it was pointed out subsequently by Wulff (1902), on the basis of his own researches on Arctic plants, that a large number of species from high latitudes possess well-developed mycorrhiza. Moreover, of sixteen species collected and investigated by this author in Spitzbergen, all were characterised by the presence of abundant sugar and the absence or scanty appearance of starch in the leaves, and exhibited a very feeble transpiration current as compared with that developed by plants in warmer climates. For these Arctic species, the most favourable conditions for transpiration appeared to be provided by temperatures about 5° C. and a relative air humidity of 60–70 per cent.

The last decade of the nineteenth century had yielded a notable accumulation of observations on the distribution, cytology and biological significance of endotrophic mycorrhiza. Stahl had put forward new views respecting the beneficent rôle of the endophytes. The observations of Magnus (1900), Shibata (1902), and Petri (1903)

had confirmed Frank's observations on the mycorrhiza of Orchids and shown that intracellular digestion with subsequent absorption of the products was not confined to this group of plants.

During the opening years of the new century the extensive researches of Gallaud (1905) pushed home these conclusions, while the improvement in microtechnique provided him with the means of explaining certain inconsistencies in the records of his predecessors, especially in respect to the organs described as "sporangioles."

Holding the view that many of the earlier observations on endotrophic mycorrhiza had been made on species showing a relatively advanced and complex condition, Gallaud undertook an investigation of cases likely to exhibit less specialised relations with the view of throwing fresh light on the biology of mycorrhiza. The work planned included the examination and description of the mycorrhiza of a large number of species, including some previously examined by Schlicht and others, a study of the structure and functions of special organs formed by the endophytes, together with observations on any reciprocal action observable between fungus and host, and the isolation and cultivation of the endophytic fungi. Included with his original observations on these matters are a useful historical review and an extensive bibliography of the literature.

Gallaud's observations on the production of special organs by the endophyte are of interest, and the structures described were found in practically all the plants examined by him. In the intercellular spaces of the root cortex he noted that the mycelium commonly formed cylindrical expansions the size and shape of which were determined by those of the individual air-spaces in which they were found. Sometimes these were replaced by terminal and intercalary swellings with thickened walls and densely granular contents, identical in appearance with the "vésicules" described by Janse and other workers. Occasionally the hyphae branched repeatedly in the intercellular spaces; more often this took place within the cells, giving rise to characteristic intracellular structures named by Gallaud "arbuscules." On casual examination appearing as homogeneous granular masses filling the cells, in thin sections the "arbuscules" could be resolved into more or less complicated branch-systems or tufts of hyphae, the ultimate branchlets of which, 1μ to 2μ in diameter, were difficult or impossible to distinguish without the aid of a careful technique and high magnification.

They were terminal or lateral upon the main hyphae and showed much variation in morphological detail, varying in size and com-

plexity of branching in different plants (Figs. 12 *a*, *b*). In certain cells "arbuscules" were replaced by granular masses often nodular in outline but without definite hyphal contours. Showing marked variability in structural details and stainability, the latter were evidently analogous to the structures described by Janse as "sporangioles" and by Petri (1903) under the name of "prosporoidi." Gallaud retained the former name without attaching any significance to them as reproductive organs of the fungus.

From his own observations he concluded that the production of vesicles was a normal phenomenon for the fungi of all endotrophic mycorrhizas except those of Orchids, and he believed them to be of the nature of storage organs. Arbuscules were present in the majority of plants examined and were regarded as haustorial branch systems functioning as absorptive organs for the endophyte; they were believed to be organs peculiar to mycorrhizal fungi.

Gallaud's interpretation of "sporangioles" as alteration products produced by intracellular digestion of arbuscules is of special interest, inasmuch as it correlated and explained the observations of previous workers and provided a reasonable explanation of the structure of endotrophic mycorrhiza in general. The evidence offered is convincing for the correctness of this view, as is that which established their identity with the "sporangioles," "Klumpen," "masses granuleuses," and "corps de dégénérescence" of previous observers. The morphological peculiarities previously ascribed to them clearly depended upon the phase of digestion observed in individual cells, the most advanced representing only residual remains of the original mycelial contents. At this stage all traces of mycelial structure had disappeared, the remains of the hyphal membranes and other residues having contracted and hardened to form a homogeneous refringent mass with definite outlines attached to the living portion of the mycelium by an empty hyphal stalk (Figs. 13 *a*, *b*, 16). The wide distribution of "sporangioles" was a measure of the prevalence of intracellular digestion in endotrophic mycorrhizas and, in common with the existence of arbuscules, was believed to provide evidence of affinity in the fungi concerned in their formation.

Excluding the mycorrhiza of Ericaceae, which was thought to approach the ectotrophic type in structure, Gallaud classified the endotrophic mycorrhizas studied by him as follows:

1. *Arum maculatum* series. Mycelium at first intracellular, later intercellular; arbuscules or sporangioles usually simple and terminal, not localised in definite layers of cells.

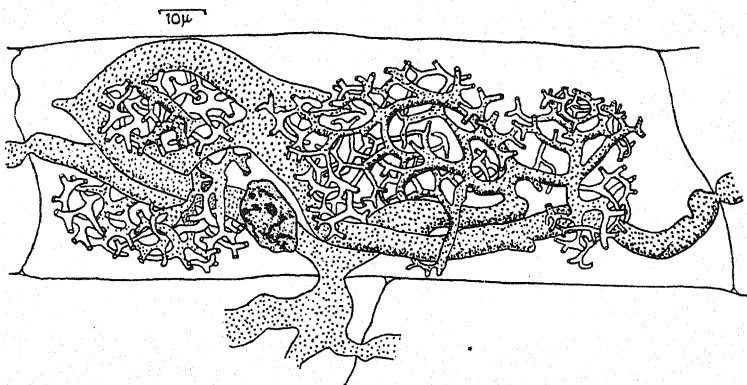


Fig. 12 b

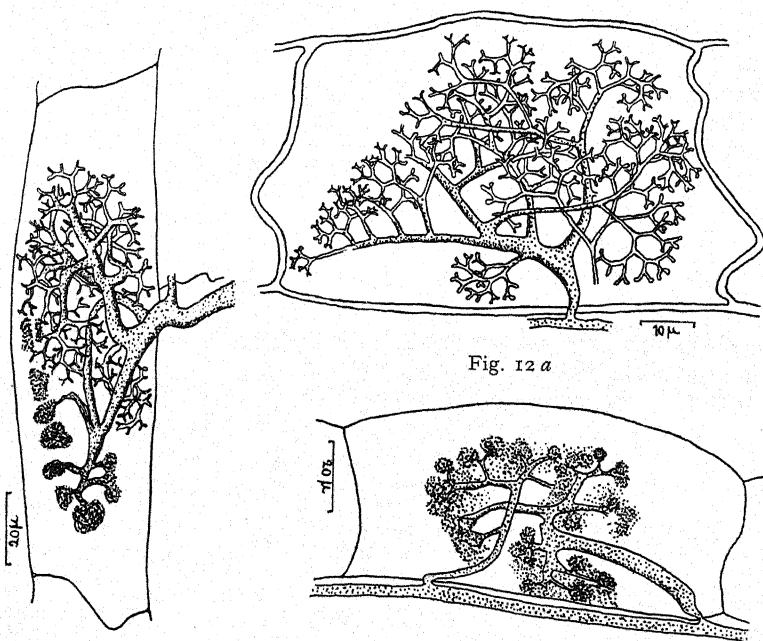


Fig. 12 a

Fig. 13 a

Fig. 13 b

Figs. 12 and 13. ENDOTROPHIC MYCORRHIZA.

12 a. *Arum maculatum*. Young arbuscule; simple type.

12 b. *Sequoia gigantea*. Young arbuscule; compound type.

13 a. *Allium sphaerocephalum*. Arbuscule in course of transformation to sporangioles.

13 b. *Ornithogalum umbellatum*. Arbuscule undergoing change to sporangioles.

(All after Gallaud, *Rev. Gén. de Bot.* 1905.)

2. *Paris quadrifolia* series. Mycelium always intracellular; arbuscules or sporangioles usually compound, not terminal, localised in definite layers.

3. *Hepatic* series. Mycelium always intracellular with arbuscules and sporangioles not localised in layers.

4. *Orchid* series. Mycelium always intracellular with differentiation to "Pilzwirhzellen" and "Verdauungszellen."

In each of these series Gallaud included plants of the most diverse affinities and habitats. For example, with *Arum maculatum* are grouped Monocotyledons and herbaceous Dicotyledons of many different families and likewise the marattiaceous fern, *Angiopteris durvilleana*. With *Paris quadrifolia*, a like heterogeneous collection of Monocotyledons and Dicotyledons together with *Sequoia gigantea*, and other Conifers, and *Ophioglossum vulgatum*. With *Pellia epiphylla* and other Hepatics were associated the prothalli of *Lycopodium* sp., and with the Orchids such diverse species as *Tamus communis* and *Psilotum triquetrum*.

It is difficult to believe that so heterogeneous a grouping has any real significance. Gallaud himself was careful to point out that his classification was based only on the morphological characters of the mycorrhizas. On critical examination he could find no reasons for believing that the differences described had any taxonomic value in respect to individual endophytes and expressed the view that all the fungi concerned could be placed in a common group:—"Les champignons endotrophes appartiennent à une même groupe dont les subdivisions correspondent aux différentes séries de mycorhizes."

The success attained by a French botanist, Noël Bernard (1903, 1904), in isolating the endophytes of *Cattleya* and other Orchids encouraged Gallaud to make similar efforts on other plants. The fungi subsequently isolated by him, e.g. species of *Fusarium*, *Mortierella*, *Alternaria*, *Cephalosporium*, etc., etc., were correctly regarded as members of the epiphytic fungus flora of the roots. The true endophytes defied his efforts, and he concluded that the intracellular digestion suffered by the mycelium had impaired its power of active growth.* His attempts to determine the taxonomic position of the endophytes by inoculation of known forms into seedlings raised under aseptic conditions were equally unsuccessful.

To the solution of the main problem—the biological relation of fungus and host in mycorrhiza—Gallaud's researches contributed little or nothing. Despite the *quasi* parasitic distribution of the intracellular mycelium and his recognition that its harmlessness

depended chiefly upon the digestive activity of the root-cells—he anticipated Bernard by comparing these cells with the phagocytes of the animal body—he concluded, in the light of his own observations, that “pour les mycorhizes d’ordre inférieur que j’ai rangées dans les séries de l’*Arum*, du *Paris* et des Hépatiques, il ne saurait y avoir de symbiose harmonique entre la plante et le champignon. Ce dernier est simplement un saprophyte d’ordre spécial, *saprophyte interne dans les racines, où le pouvoir digestif des cellules, sans entraver son développement, empêche qu’il ne leur cause des dommages importants.*” And elsewhere—“On ne peut donc dire qu’il y a symbiose harmonique entre les deux plantes mais bien plutôt *lutte entre le champignon envahissant, mais peu nocif, et les cellules* qui se défendent grâce à leur *puissance digestive.*”

Gallaud’s researches followed the conventional lines of investigation laid down by earlier workers. In certain respects, however, his work on mycorrhiza marks the beginning of a new period. More especially in relation to the identification of the endophytic fungi was a changed point of view noticeable: the need for experimental verification was noted, and the absence of any satisfactory proof of the identity of the fungi isolated was frankly admitted. In respect also to the biological relation between vascular plant and fungus, Gallaud showed a disinclination to accept traditional hypotheses, and his own views indicated the necessity for experimental investigation of a more critical kind than had hitherto been bestowed upon mycorrhiza problems.

Although published in 1905, Gallaud’s memoir had appeared earlier in the form of a thesis and it is due to this fact that he left unnoticed a paper published at the end of 1904 (Bernard, 1904 a) that was destined to bring about a profound change of opinion with regard to the biology of Orchid mycorrhiza.

Two years earlier, in 1902, there had appeared a paper on tuberisation by a French botanist, Noël Bernard, followed in 1903 and 1904 by other communications giving an account of the author’s researches on Orchid mycorrhiza. Publication of these papers marks the beginning of a new and fruitful period of investigation on mycorrhiza. In the first, Bernard recorded the isolation of members of the genus *Fusarium* from the roots of various Orchids, believing at that stage of his work that these were the true endophytes. He recorded also the new and remarkable observation that the presence of the endophytic fungi was essential for successful germination of the seeds of various Orchids, and directly related the tuberous habit

of the young Orchid embryo with infection by the specific root fungus at germination. He applied the knowledge gained experimentally to explain the development of the tuberous habit in other Orchids, in *Ranunculus ficaria* and in Potato, and elaborated an entirely original theory of tuberisation which will be considered at greater length in a later chapter. Bernard's classical researches on the mycorrhiza of Orchids will now be considered in detail.

CHAPTER V

Orchidaceae: Bernard's discovery of the obligate relation in Orchids; the pathogenic theory of infection—Burgeff—Recent researches on the germination of Orchid seeds: Knudson; Bultel; Wolff—The obligate character of infection: Constantin and Dufour; Huber—The symbiotic and asymbiotic methods of germination and their practical applications.

Noël Bernard's experimental studies initiated the modern period of experimental enquiry and mark an epoch in the history of research on mycorrhiza. He approached the subject with a point of view essentially different from that of his predecessors, and, almost at once, reached conclusions respecting the biological relation between fungus and host plant so novel and stimulating that they illumined the subject as a whole and produced a profound effect upon the point of view of subsequent investigators.

To Bernard, the root fungi of the Orchids were parasites, attacking the embryo plantlet at the earliest stage of growth and persisting in the adult as a chronic—although relatively benign—condition of disease. He regarded the Orchids—"comme les plantes atteintes d'une maladie parasitaire chronique qui commence à la germination et persiste en général jusqu'à l'état adulte; maladie bénigne en un certain sens, puisqu'elle n'empêche pas la vie, mais qui ne constitue pas moins une tare physiologique des Orchidées en général, une condition de nature à faire comprendre quelques-unes des anomalies de ces plantes qui passent pour singulière aux yeux mêmes d'observateurs peu initiés à la Biologie végétale." (Bernard, 1904 *a.*)

Bernard's earlier observations were made upon the germination of seeds of *Neottia* and other Orchids, and dealt also with the possibility of correlation between the modes of development of different species and their infestation by the fungal endophytes. A study of the Ophrydeae from this point of view focussed his attention upon the periodicity of tuber-formation in members of this group and its relation to fungal infection of their roots, whence he was led to speculate upon the physiology of tuber-formation in general and to put forward and, subsequently, elaborate an hypothesis to

explain the phenomena of tuberisation (Bernard, 1899, 1900, 1902 a, b, c, 1903).

This theory, together with its later developments, is dealt with fully elsewhere in the present work, so leaving the way clear for an account of the experimental researches on Orchids and a critical review of the theories based upon them.

As is well known, the seeds of Orchids are extremely small and light and are produced in immense numbers. Darwin (1862) had estimated the number of seeds in a single capsule of some of the common British species at over six thousand and this number is known to be greatly exceeded in some of the tropical species.

At the beginning of the nineteenth century it was commonly believed that they were incapable of germination. Salisbury (1804) was the first to announce that he had observed germination, and he figured the seedlings of *Orchis morio* and *Limodorum verecundum*. The seeds are of very simple structure, each consisting of a small group of similar cells enclosed by a thin reticulate seed-coat (Figs. 14, 18). In certain genera the cells are larger at the suspensor end of the seed; otherwise there is no differentiation of tissue and no distinction into root and shoot such as exists in the embryos of most plants. If removed from the capsule and sown on an ordinary moist substratum away from the parent plant or other Orchids, their behaviour varies with the genus of Orchid. Seeds of *Epidendrum*, for example, undergo practically no change; those of some other genera, e.g. *Odontoglossum*, increase slightly in size and develop chlorophyll; those of *Cattleya* may continue to grow for several months, forming a minute green tuberous structure with rudimentary hairs—the primary tubercle or “protocorm,” while in rare cases, as shown by Bernard for the genus *Bletilla*, the seedling may develop a slender stem with foliage leaves. In all cases, if supplied only with the formal conditions for germination, at a critical stage of development varying with the genus of Orchid, growth comes to an end and the seedlings perish.

Bernard's early experience had brought him into touch with the difficulties encountered by practical Orchid growers, many of whom had devised empirical methods to facilitate the successful raising of Orchids from seeds. For example, sowings made upon moss laid upon the pans containing the parent plants, special attention being paid also to conditions such as temperature and moisture. In spite of every precaution suggested by practical experience, the raising of Orchids from seed continued to be a somewhat precarious adven-

ture, leaving growers quite in the dark as to the real nature of the critical factors which controlled germination. Practical experience had shown also that the difficulty of raising seedlings varied greatly with the genus and species of Orchid. Seeds of *Cattleya* and *Cypripedium*, for example, were relatively easy to germinate successfully, while those of *Odontoglossum*, *Phalaenopsis* and *Vanda* presented the greatest difficulty. The irregularities of behaviour observed in horticultural practice were confirmed by Bernard's earlier experiments, which, moreover, failed to provide any consistent explanation of the inconstant results obtained. He noted, however, that the methods of cultivation used in the greenhouses of successful growers must involve the presence of the root fungi in the soil about the roots and eventually their acclimatisation in the plant houses. Hence, he argued, an explanation of the fact that germination of seed, at one time believed to be impossible, had been made practicable by modern conditions of culture, and a justification of the popular method of sowing seed in close proximity to the parent plants. The fact that he had failed to secure, under aseptic conditions, germination of seeds of various Orchids commonly raised by growers, in conjunction with the nature of the successful methods adopted by the latter, had indeed convinced Bernard that the presence of the endophytic mycelium was essential for successful germination, and it became his immediate concern to obtain experimental support for this view.

In the first comprehensive account of his work, published in 1904, his objectives were stated as follows:

1. To germinate Orchid seeds under strictly aseptic conditions on sterilised media suitable for the cultivation of the plants.
2. To isolate and identify the endophytic fungi in pure cultures.
3. To compare the behaviour of infected and uninfected seeds for each species of Orchid.

Aseptic seeds were obtained by careful selection of ripe capsules before dehiscence, external sterilisation of the fruits by means of formalin, and transference of the seeds with aseptic precautions to tubes containing gelatine slopes of a decoction of salep¹.

The record of Bernard's first successful attempts to isolate and establish the identity of the Orchid endophytes has historical interest and merits a brief description. The first was made with seeds of a

¹ The "salep" of commerce is obtained by reducing to powder the dried tubers of various oriental species of *Ophrydeae*.

hybrid *Cattleya* removed aseptically from a ripe fruit and placed to germinate, some under aseptic conditions in the laboratory, the remainder in the greenhouse of a professional grower as in ordinary horticultural practice. Two months later the aseptic seed cultures were almost unchanged, while those in the greenhouse were germinating and the seedlings showed active fungal infection of the suspensor region. Some of the infected seedlings were transferred to tubes of gelatine media and gave a mixed growth of mycelium, the fungi responsible for which were individually isolated, and tested by inoculation into the aseptic seed cultures. One of the forms thus secured at once provoked germination of a normal and regular kind and was observed to cause characteristic fungus infection, thus providing the necessary proof of its identity with the endophyte.

For the second attempt the fruit of a hybrid *Cypripedium* was available. In this case the endophyte was isolated, not from seedlings, but from fragments of the root of one of the parents (*C. insigne*), the fungal growth from which yielded a form identical morphologically with that obtained from *Cattleya*, and correspondingly effective in provoking the germination of aseptically sown seeds of *Cypripedium*. On the other hand, attempts to establish the identity of a fungus isolated from the roots of *Spiranthes autumnalis* by inoculation into seed cultures of the same species were not successful, thus indicating that the presence of the specific endophyte was not the only critical factor in successful germination.

Bernard was impressed by the difficulty he experienced in isolating the true endophytes from pieces of heavily infected tissues, attributing the relative infrequency of mycelial growth from within to the efficiency of the intracellular digesting mechanism—"en général l'endophyte n'est en voie de croissance active que dans un petit nombre de cellules aux limites des régions infectées. . . ."

The two successful isolations just described are notable as the first in which the identity of a root-fungus was definitely proved by inoculation into seedlings raised under aseptic conditions. They are also of interest as the first experiments providing proof of a definite physiological relation between the endophytes and their vascular hosts, a relation predicted by Bernard in an earlier paper published in 1902.

The fungi isolated from *Cattleya* and *Cypripedium* formed spore-like structures freely in culture and were referred provisionally to the genus *Oospora*, Bernard's earlier attribution of the Orchid endophytes to the genus *Fusarium* being withdrawn as an error

of observation due to the prevalence of the members of the latter genus in the epiphytic flora of the roots, and the relatively slight activity of the true endophyte in isolation cultures.

The behaviour of seeds of *Cypripedium* under controlled conditions in the presence of the endophyte was described by Bernard as follows.

Germination was initiated by penetration of the large cells of the suspensor end of the seed, whence the mycelium spread from cell to cell of the middle and outer parts of the embryo which increased rapidly in size and ruptured the seed-coat. At this stage the embryo of *Cypripedium* resembled that of *Neottia* in respect to the precocious appearance of abundant starch in the tissues, the lack of chlorophyll and the absence of root-hairs. Within the inner zone of infected tissue, the cells soon showed the cytological features characteristic of digestive activity, i.e. clumping of the hyphae, hypertrophy of the nuclei, and the eventual appearance of a structureless mass of mycelial residues. In the outer zone of tissue, the mycelium continued to invade fresh cells. With the spread of infection, starch disappeared from the invaded cells, and a terminal meristem, separated from the infected region by a zone of active growth, was formed.

A second stage in development was characterised by the formation of chlorophyll and development of root-hairs involving a sharper delimitation of the infected area. The growing axis remained immune from attack, the mycelium underwent digestion in all the cells of the primary tubercle, and the zone of active infection became restricted to a region between the original point of infection and the apical meristem. The close of this period in seedlings about four months old was marked by the production of the first root. It is of interest to note that the young roots suffered infection, not from the mycelium-filled cells of the primary tubercle, but directly from the soil after emergence. The final stage of germination was marked by a shrinkage of the infected area and elongation of the axis with the formation of leaves and roots, following upon which the seedling took on the appearance and structure of the adult plant.

When cultured with their respective endophytes, the seeds of various other Orchid genera behaved in a manner more or less similar to that just described for *Cypripedium* (Fig. 14). Those of *Bletilla hyacinthina*, an Orchid nearly related to *Cattleya*, responded very differently. The embryo of this species at first enjoyed almost complete immunity from infection and in aseptic culture developed

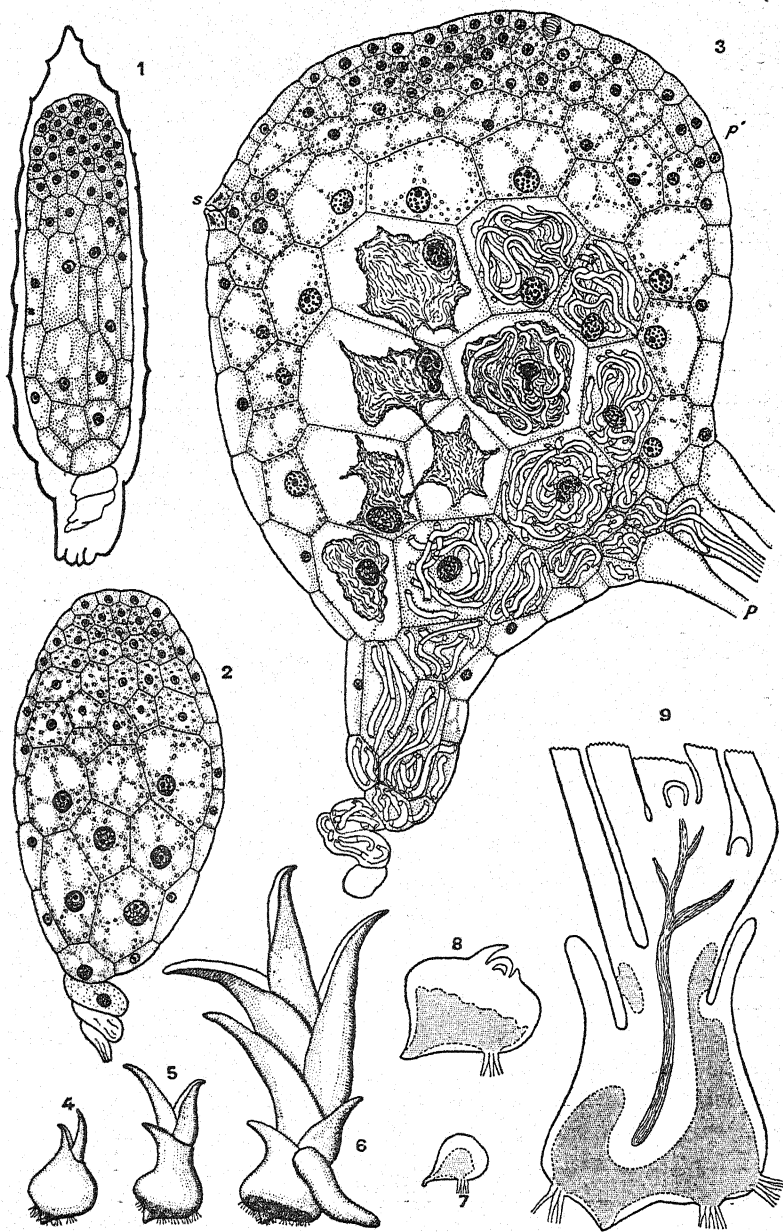


FIG. 14. Seed and Seedlings of *Odontoglossum* hybrids: 1, median longitudinal section of ripe seed showing seed-coat, embryo and remains of suspensor; 2, median longitudinal section of embryo four months after sowing, without fungus; 3, median longitudinal section of seedling, one month after infection by *Rhizoctonia lanuginosa*, s, stoma, p, absorbing hairs, p', cells divided tangentially, from outer of which absorbing hairs will develop; 4, 3 months' seedling; 5, 5 months' seedling; 6, 7 months' seedling; 7, 8, 9, median longitudinal sections through seedlings of various ages to show extent of infected region in each case (infected tissue shaded). (From Bernard, 1909 a.)

independently to a small plantlet consisting of a slender axis with absorbing hairs and a few green leaves. There ensued a critical stage during which immunity disappeared and, subject to infection by the appropriate fungus, development proceeded normally. Failing such infection, growth came to an end.

Bernard's observations on the cytology of digestion in the embryos and roots of Orchids agreed with those of Magnus and Shibata, but his interpretation of the significance of the changes which took place differed remarkably from that of these observers. Sooner or later, the cytoplasm and skeins of mycelium in the "Verdauungszellen" became reduced to amorphous masses of degenerating material enveloping the nuclei. The nuclear changes accompanying this process, regarded by Magnus and his followers as signs of increased activity, were interpreted by Bernard as signs of degeneration. According to the latter, the infected cells formed, at this stage, a dead tissue:—"Dans la lutte entre le parasite et la cellule qu'il envahit, les deux adversaires ont fini par succomber"!

To summarise briefly the important facts put on record by Bernard in these earlier observations.

Fungal species of similar morphological character were isolated from Orchids belonging to the genera *Cypripedium*, *Cattleya* and *Spiranthes*. Seeds of species of *Cypripedium*, *Cattleya*, *Laelia*, *Brassavola*, and *Bletia*, sown under strictly aseptic conditions, gave normal germination, the seedlings showing typical infection when mycelium of one or other of the endophytes was added to the cultures. Normal growth and development of the seedlings followed upon invasion of certain tissues of the embryos; in the absence of infection, either the seeds did not develop at all, or they did so very imperfectly.

The cases observed were grouped by Bernard in three categories. In the first was placed the genus *Cypripedium*, the seeds of which did not develop at all under aseptic conditions; in the second, *Cattleya*, seeds of which survived for several months in the absence of infection, increased considerably in size, showing a certain degree of tissue differentiation, but, lacking infection, developed no further; finally, there was the interesting case of *Bletilla hyacinthina*, a species with relatively highly differentiated seeds, representing, in Bernard's view, a condition rare among Orchids. From this preliminary work it was concluded that the effect of infection was to stimulate growth, the stimulating effect being invariably manifested in cells remote from the point of infection.

Subsequent observations have shown that, while in experimental cultures the addition of the appropriate mycelium may be delayed until the critical stage in development, under natural conditions infection of the embryo probably takes place much earlier (Fig. 19). In a majority of Orchids, the spread of infection in the embryo is strictly localised, the apical meristem remaining immune to invasion, and the roots showing complete freedom from fungal infection until they reach the soil (Figs. 19, 20, 21). A similar temporary immunity is enjoyed by the annual roots of the tuber-forming terrestrial Orchids, e.g. members of the Ophrydeae. In *Neottia*, the non-chlorophyllous habit is associated with heavier infection; mycelium is more profusely distributed in the embryo and becomes continuous throughout the vegetative tissues of the mature plant. Bernard found seeds of *Neottia* germinating in nature and observed that "elles étaient toutes plus largement infestées encore que les germinations d'Orchidées que j'ai vues dans les serres." He made also the interesting observation that the inflorescences of *Neottia* were sometimes unable to pierce through the overlying humus in which the plant grows. Under such circumstances mycelium spread from the rhizome through the tissues of the inflorescence axis and infected the seeds as they germinated *in situ*.

The isolation of a number of fresh endophytes, with continued study of their characters and relations with different Orchid species, together with the publication of his results, occupied Bernard during the years 1904-1906 (Bernard 1904 b, 1905, 1906).

He obtained experimental demonstration of the existence and maintenance of a remarkable reciprocal balance between fungus and Orchid plant. Using seeds of the same Orchid species and mycelium from the endophytes of three different genera, including the parent species, evidence of a surprising degree of specificity on the part of the latter was obtained as follows.

Germinating seeds of a species of *Phalaenopsis* were inoculated from pure cultures of the fungi isolated from *Cattleya*, *Phalaenopsis* and *Odontoglossum* respectively.

In the case of the first the mycelium invaded the seed tissues, no intracellular digestion was observed, and the mycelium parasitised and killed the seed without effecting germination. With the fungus from *Phalaenopsis*, infection took place in the usual way, and was kept within bounds by the digestive activity of the cells of the embryo; normal germination of the type described for *Cypripedium* followed upon infection. Using the fungus from *Odontoglossum*, the

intracellular digestion subsequent to infection was excessive, and germination stopped short at a stage marked by the development of root-hairs from the rudimentary axis.

Interpreting these facts in terms of his own theory of infection as a pathogenic condition of relatively slight importance to the Orchid plant, Bernard observed: "à un point de vue théorique, il résulte de ces constatations que l'état dit desymbiose est en quelque sorte un état de maladie grave et prolongée, intermédiaire entre l'état des plantes atteintes d'une maladie rapidement mortelle et celui des plantes qui jouissent d'une immunité complète."

During the same period he perfected a refined and beautiful technique for isolation of the endophytes by direct removal of a young mycelial complex of hyphae from cell to culture dish, thus eliminating the tedious and unsatisfactory procedure involved by isolation of the endophytic mycelium from a mixed growth of fungi associated with the outside of the roots (Bernard, 1909 *a*). He learned also that the endophytes of different Orchids exhibited a well-marked specificity in relation to their individual hosts and further, that the application of his experimental knowledge to the practical problems of Orchid raising was not without its special pitfalls.

Bernard alluded to these difficulties in an address delivered during an International Horticultural Exhibition at Ghent (Bernard, 1908). Three years previously, he had, by request, supplied certain French Orchid growers with *old* cultures of the endophytes from *Cattleya* and *Cypripedium*. The use of this mycelium for inoculation into seed cultures was unsuccessful and gave lamentable results, an observation confirmed by Bernard himself in the laboratory. The reason for this unexpected failure he believed to be due to the use of fungus cultures, which, by long cultivation outside their respective hosts, had lost their power of inducing germination: they were compared directly with the "attenuated strains" of pathogenic bacteria used by Pasteur in the preparation of curative vaccines. Bernard proceeded to confirm this view experimentally by similar methods to those used by bacteriologists, i.e. by re-cultivation of the "attenuated forms" within their specific host plants, and obtained results which led to the belief that complete restoration of the stimulating effect upon germination depended upon the duration of this "restorative" period in relation to the degree of attenuation existing in the first instance.

In the same address, it was suggested that the addition of fragments of the parent roots to the seed bed might facilitate germination

under horticultural conditions, the attention of his audience being drawn to the importance of selecting roots containing active mycelium.

Bernard realised that the full fruits of his discovery could only be made available to practical growers by the provision of a specially equipped laboratory, with a constant supply of infected seed cultures in full development upon which to draw for suitable strains of the fungus. In the address just mentioned he observed: "J'ai la conviction profonde que les laboratoires horticoles existeront un jour, mais la symbiose de la science pure et d'horticulture pratique est trop encore dans l'enfance pour que leur utilité s'impose aux esprits."

As he extended his acquaintance with the Orchid endophytes, he modified his earlier views respecting their systematic position. Finally, in view of the marked resemblance of the mycelium to that of *Rhizoctonia violacea*, a fungus common on Potato, he placed them definitely in the genus *Rhizoctonia*. Although impressed by the general similarity of all the forms extracted—"ces champignons sont moins variés que les plantes desquelles ils proviennent"—he gave specific rank to three forms differing in respect to habit and minor morphological details; namely, *Rhizoctonia repens* isolated from *Laelio-Cattleya*, *Cypripedium* and a number of other genera; *Rhizoctonia mucoroides*, from *Phalaenopsis* and *Vanda*; and *Rhizoctonia lanuginosa* from one species of *Odontoglossum*. The forms included under *Rhizoctonia repens* were obtained from many common European Orchids, and proved also to be relatively the most abundant in cultivated species. It may be inferred, therefore, that these fungi have a very wide geographical distribution in nature. All the endophytes doubtless occur in the soil or detritus about the roots of Orchids, but there is at present no exact information as to the occurrence of fungi belonging to the *Rhizoctonia* group in soil.

The mycelium of the endophytes showed a characteristic mode of growth within the tissues, spreading from cell to cell and forming in each a tangled skein of twisted filaments. In Bernard's cultures, a similar tendency to form skeins or "pelotons" was observable, but the habit was not regarded as specially characteristic since it had been observed also in other members of the Mucedineae, the group in which *Rhizoctonia* was included.

When associated with the Orchid plants, spores or other reproductive structures were not produced by these endophytic fungi, but in old cultures the mycelium gave rise to filaments of short segments resembling conidia, and in certain forms these branched hyphae

anastomosed to form small yellow or brownish sclerotia, the production of which is characteristic of the genus *Rhizoctonia* as of many other Fungi (Fig. 15).

A few words may be added to the accounts already given describing infection in the roots and rhizomes of Orchids. In general, the distribution of mycelium is constant for any one species, but varies in different genera. Hyphae usually enter through the root-hairs but may invade cells of the piliferous layer directly. They pass through the "passage cells" of the exodermis, when this layer is present, and traverse the outer tissues of the cortex in a more or less radial direction—in one species, *Lecanorchis javanica*, Janse described the infecting hyphae as fusing to form a flat mycelial ribbon—and eventually give rise to a zone of typical infection by the formation of the characteristic skeins or "pelotons" within the cells. The exact position and extent of this infected tissue vary in different genera; in some it is restricted to two or three layers of cells in the middle region of the cortex, in others it occupies almost the whole of the cortical tissue. As infection is localised in the embryo, so in roots and elsewhere it is restricted to the cortex and is absent from meristematic tissues and from those of the vascular cylinder enclosed by the endodermis. In some cases mycelium is confined to localised areas of the root, and occasionally roots may be found entirely free from mycelium, especially in certain genera. Like other chlorophyll-containing organs, aerial roots are usually immune to infection but they may suffer invasion if in contact with the soil and lacking chlorophyll. In the case of individual cells, immunity is indicated, not only by the possession of chlorophyll but also by the presence of special contents—raphides and other crystals, tannin and the like.

The rhizomes of non-chlorophyllous species and of certain green forms show a distribution of mycelium similar to that in roots, and, in general, the tissues of these plants are subject to heavier infection.

Intracellular mycelium in the active condition can best be observed in the tissues nearest to the growing regions; in older parts a majority of infected cells show the mycelium in the "clumped" condition or in various stages of disintegration. As already noted, Magnus described a differentiation of infected cells as "Verdauungszellen" (digesting cells) in which the hyphae were destroyed by the digestive activity of the host cells, and "Pilzwirhzellen" (host cells) in which they persisted, retaining their vitality throughout the resting period and serving as centres for re-infection when vegetative activity was resumed (Figs. 22, 23). Magnus attached great importance

to this differentiation and to the arrangement in space of the two kinds of cells in *Neottia*, and even suggested the possibility of a classification of Orchids based upon these characters.

Subsequent work has rendered it difficult to maintain the belief that such a regular arrangement exists, even in *Neottia*, and has proved that "host cells" are not present in many genera of Orchids, all those infected being subject to eventual digestion. It is probably safe to assume that the characters shown by the mycelium in the so-called "host cells" represent a relatively early stage of infection preceding the onset of digestive activity.

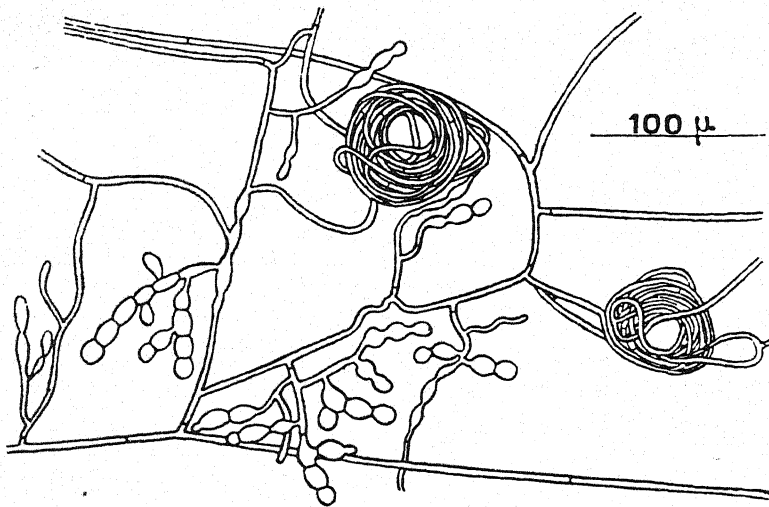


FIG. 15. *Rhizoctonia repens*: mycelium from pure culture. Note chains of conidia-like segments and "pelotons." (From Bernard, 1909 a.)

Certain isolated observations of physiological interest were recorded by Bernard. One related to the marked ability of the endophyte to digest cellulose, another, made several years later, to the fungicidal action exerted by tissue extracts of the tubers of members of the Ophrydeae, these organs of the plants being ordinarily immune to infection (see p. 93).

Of still greater interest was the observation that the stimulus to germination normally provided by the endophytes could be replaced by raising the concentration of organic substances in the substratum used for aseptic seed cultures, i.e. as pointed out by

Bernard, by a procedure directly comparable with that known to provoke the development of virgin eggs: "L'action des champignons sur les embryons d'Orchidées peut sans doute être comparée à l'action des spermatozoïdes sur les œufs" (Bernard, 1909 *b*).

These experimental researches on Orchids not only yielded a rich harvest of new and proved facts, but they drew attention to the necessity and value of exact experimental methods in this field of work, and brought the technique of the bacteriologist to the service of the worker at mycorrhiza problems.

As experimental data accumulated, the biological aspects of the symbiotic relation in Orchids and their relation with the phenomena of parasitism, immunity, and pathogenic infection tended more and more to monopolise Bernard's attention. In his most comprehensive work, *L'Évolution dans la Symbiose* (1909 *a*), he elaborated his theory of pathogenic infection, illustrating and supporting his argument by reference to his own experimental results. Another paper dealing with the phenomena of immunity in relation to symbiosis was published in the same year (1909 *b*).

Space does not permit of more than a brief reference to the many interesting facts and reflections brought together in these papers, in which the author sought more especially to study the evolution of the symbiotic habit in plants, and the conditions regulating the "balance of power" between the constituent organisms.

Reviewing the remarkable phenomena of development subsequent to seed infection and noting the obligate character of the association in Orchids, he expressed the opinion that the mutual adaptation of Orchid plant and fungus was a phenomenon linked up with that of the origin of species. Comparative study of the developmental behaviour of different species suggested that the direct ancestors of Orchids were herbaceous perennials with normal germination, the formation of an embryonic tubercle—"protocorme"—having been acquired as a direct consequence of fungal infection. Incidentally, Bernard attached great theoretical importance to the formation of this embryonic structure, in view of its resemblance to the juvenile stage of species of *Lycopodium* and other Pteridophytes.

On the other hand, he held that the relation of germination to fungal infection was not a consequence peculiar to the latter, but could be produced with equal efficacy by the action of concentrated organic solutions. His own experiments had shown—and in view of modern work upon non-symbiotic germination of Orchids it is worth recalling this—that it was easier and more certain, under

experimental conditions, to effect the germination of certain Orchids by the action of concentrated organic solutions than by that of the specific endophytes, of which it was often difficult to secure strains in a sufficiently active condition.

Discussing the significance of symbiosis in relation to the evolutionary history of Orchids, Bernard emphasised the comparative ease with which certain species could be raised from seed by symbiotic methods: "il a été relativement facile de faire germer les Orchidées sans champignon, et il semble bien qu'il faudrait seulement du temps et quelques soins pour prolonger leur culture dans ces conditions" (Bernard, 1909 *a*).

Whatever might have been the significance of symbiosis in the evolutionary history of plants other than Orchids, it was held by Bernard that the habit had played an important part in that of the latter group. The exceptional features shown by *Bletilla hyacinthina* and its near relatives he regarded as rarely preserved characters, representing the nearest approach to the ancestral condition and the starting point of an evolutionary series among living Orchids, of which the last terms were represented by *Cypripedium* and similar types in which the earliest stages of development had become directly dependent upon the nutritive stimulus ordinarily supplied by symbiosis with the appropriate endophyte. In his view, the Orchids represented a remarkable case of adaptation to parasitic invasion, their survival as a group pointing to the possible disappearance of other forms, both plant and animal, which had succumbed to similar attack.

Bernard's later researches were carried out at the Pasteur Institute, a circumstance possibly not entirely unconnected with the fact that he became more and more impressed by the close analogy between symbiotic phenomena and those revealed by the work of Pasteur and Metchnikoff on pathogenic infection: "Dans une étude de la symbiose les expériences de Pasteur doivent servir à éclairer les théories de Lamarck et de Darwin" (Bernard, 1909 *a*). To him the relationship in Orchids represented a condition on the threshold of disease offering an exceptional opportunity for the study of vegetable pathology. The digesting cells of the roots and embryos were directly comparable with the *phagocytes* of the animal body, and the process of digestion with a *phagocytosis* which constituted the chief weapon of the Orchid plant against parasitic invasion of its tissues.

The views expressed by Frank, Shibata and others, who had seen

in the phenomena of digestion in the root-cells a mechanism by which mycorrhiza plants obtained a supply of nitrogenous food material from their invaders, were not acceptable to Bernard. This "curious hypothesis," as he described it, was not in his view supported by the observed facts, and was indeed improbable in view of the reactions shown by the cells of Orchid embryos to infection, whether in a condition of balanced symbiosis with the appropriate fungus, or, when suffering damage from invasion by a too virulent strain from another species of Orchid. The formation of special haustorial branches or "arbuscules" by many endotrophic fungi and the conversion of these into structureless "sporangioles" was held to point to a similar conclusion.

Comparison with the mechanism of resistance and immunity in animals was pressed to its limits by Bernard. For example, he claimed that seed cultures inoculated by a strain of *Rhizoctonia* too weak to effect germination, became incapable of development when subjected to the action of a suitable strain—they had been "vaccinated" and so rendered immune to the action of the more "virulent" form—a strange reversal of the result desired in ordinary vaccination!

The *obligate* condition in Orchids was held to be associated directly with a secondary effect of infection, viz. a rise in the concentration of the cell sap—in his view, equally well brought about under experimental conditions by chemical means:—"Le phénomène de croissance est la manifestation visible de transformation du contenu de la cellule ayant pour résultat d'augmenter la quantité absolue de substances dissoutes dans le suc cellulaire" (Bernard, 1904 a). While he admitted the possibility of autonomous existence for certain species in the adult state, Bernard knew of no case of Orchid germination lacking fungus infection in nature or horticultural practice, although such had been observed experimentally.

Discussing the possible existence of species immune to infection in the adult state, he recalled Frank's observations claiming immunity for roots of *Epipactis latifolia* and *Listera ovata*, the view of Johow respecting *Wulfschlaeglia* and that of MacDougal for *Cephalanthera oregana*. A similar temporary freedom from infection in mycotrophic plants other than Orchids had been noted by himself in *Psilotum triquetrum*, and by Gallaud (1905) in *Ranunculus ficaria* and *Arum maculatum*. In none of these cases, it was pointed out, had any experimental evidence been offered that *germination and development* could take place independently of infection. From his

own observations on *Epipactis*, he thought it unlikely that such would be forthcoming. Of interest in the same connection may be noted again the partial and intermittent immunity shown by a majority of Orchids, as exemplified by the complete freedom from infection of certain tissues, e.g. meristems, chlorophyllous cells and the tissues of the vascular cylinders, and by the progressive localisation of infection in the tissues of germinating embryos. Moreover, in some species, e.g. *Neottia*, fungal infection was found to be extensive and continuous throughout life; in others, e.g. members of the Ophrydeae, it was periodic and intermittent, the roots of each season's growth being newly infected from the soil after emergence. Bernard was concerned to find a physiological interpretation of these facts and, in a posthumous paper, described the fungicidal effects observed in the tuber tissues of members of the Ophrydeae (Bernard, 1911).

His early observations on this group of Orchids had shown that the tubers were immune from infection. Experimentally, it was found that small pieces of tissue, removed aseptically from a living tuber and imbedded in culture vessels of gelatine nutrient inoculated with one or other of the endophytes, caused arrest of growth and death of the protoplasm, as the mycelium came within range of substances diffusing from the fragments of tuber tissue. The fungicidal effect was produced only by living tissue, the lethal substance being destroyed by temperatures above 55° C. Extending these observations, he reached the conclusion that there was produced, in tubers of the Ophrydeae, a fungicidal substance, easily diffusible and readily destroyed by heating, the action of which was specific upon the endophytes and varied with the forms extracted from different species of Orchid.

Bernard's results have since been confirmed by Nobécourt (1923) and by Magrou (1924). The former found that the action of the tuber was inhibited, not only by heating, but also by chloroform and by cooling to 15° C. He believed that the fungicidal substance was a secondary product formed by the interaction of toxins from the mycelium with substances diffusing from the tuber tissue. Bernard had been prepared to regard the effect as due to an "antibody" secreted by the intact tuber under the influence of an "antigen" diffusing from the mycelium present in the roots. Magrou's recent experiments afford confirmation of the latter view by showing that diffusion from the fragment of tuber, before inoculation by the fungus, is sufficient to render the gelatine medium toxic to mycelial growth.

Noël Bernard's brilliant researches were brought to a premature end by his untimely death in 1911. He had initiated a new line of experimental investigation and made it peculiarly his own. Already, in one short decade, he had changed the whole aspect of the mycorrhiza problem, bringing it into touch, on the one side with the physiology of parasitism and pathogenic infection, and, on the other, with the difficulties experienced by practical horticulturists. The knowledge acquired by experiment in the laboratory was placed at the service of Orchid growers and, in the hands of some of them, has since proved to be of great practical value. He left to his colleagues and successors the task of completing his work; in particular, of providing the requisite experimental support for his novel and stimulating theory of tuberisation.

His researches had already given an impetus to research on mycorrhiza, and the success of his experimental work on Orchids was doubtless indirectly responsible for the publication of a monograph entitled, *Die Wurzelpilze der Orchideen*, in which Burgeff (1909), a German worker, contributed an account of his own experiments, confirming many of Bernard's results and extending the latter's observations in certain directions.

Burgeff expressed the view that the endophytes belonged to a single group of Fungi, the Orcheomycetes, for which he proposed the generic name *Orcheomyces*, without reference to the systematic affinities of the genus. He added to the list of root fungi already isolated by Bernard and classified the known forms in five groups, each including a number of forms to which he gave specific rank; he contributed also additional details as to the specificity of forms isolated from particular Orchid species. Devoting special attention to the physiology of the root fungi in pure culture, he put on record a number of new observations bearing on their metabolism, of which perhaps the most significant related to their inability to grow on substrata lacking combined nitrogen, and hence, to the absence of any positive evidence for the fixation of atmospheric nitrogen. In view of the recent researches of Wolff (1925) on the endophyte of *Neottia*, it is clear that this conclusion may require revision (see p. 107).

Burgeff was unable to confirm Bernard's observation that the efficacy of the endophytes in respect to germination was impaired by prolonged cultivation outside their host plants, finding, on the contrary, that a culture 26 months old showed no decline of activity when used for the inoculation of aseptic seed cultures. He believed the activating effect of the mycelium upon development to be due

to the production of enzymes which acted upon the carbohydrates present in the seed tissues and thus expedited germination. Bernard had related the stimulus following infection to a rise in concentration of the cell sap. On Burgeff's hypothesis, this would follow from an increased sugar content, due to the diastatic activity of hyphae in contact with the starch-containing cells of the embryo and roots, thus accounting also for the observed disappearance of starch from these tissues subsequent to infection.

Burgeff was Stahl's assistant, and this monograph on the Orchid fungi was doubtless prompted in part by a desire to relate the new facts established by Bernard to the older theories of nutrition put forward by Stahl and others. On theoretical grounds he found himself in general agreement with Stahl in respect to the existence of a symbiotic relation beneficial to the vascular plant. His researches, like those of his predecessor Bernard, were brought to an end by the untimely death of the observer.

To Kusano (1911), a Japanese observer, is due the recognition of an unusual and remarkable type of mycorrhiza in *Gastrodia elata*, a curious non-chlorophyllous Orchid from Japan. The species is described as widely distributed in Japan, occurring chiefly in woods of *Quercus serrata* and *Q. glandulifera*. It had been mentioned earlier by Johow (1889) as a humus saprophyte with mycorrhiza, but, previous to the publication of Kusano's paper, nothing definite was known respecting either the type of mycorrhiza or the mode of nutrition.

The plant appears above ground only in the flowering stage, the vegetative body consisting of a system of subterranean rhizomes and tubers bearing scale leaves. The large flowering tubers are 10 cm. to 17 cm. in length and give rise to lateral offsets in the form of small daughter tubers. At the end of May the flowering tuber produces an immense inflorescence a metre or more in height. Externally, the massive tubers are covered with a corky skin like that of a potato, enclosing a parenchymatous tissue with small centrally placed vascular strands. In the autumn, the parenchyma contains abundant carbohydrate reserves, the chemical nature of which was described in detail by the author.

Kusano's interest in the plant was first aroused by observing the relatively small proportion of flowering tubers as compared with the number of offsets produced, and also by noting the freedom of the latter from any trace of fungus infection. Of mycorrhiza, in the strict sense, there was none, since the plant is rootless.

The offsets showed no signs of fungus infection, but the flowering tubers were found to be invariably associated with the rhizomorphs of *Armillaria mellea*, the Honey Agaric. This fungus, known to foresters in Europe as a most destructive parasite, was described by Kusano as common on the older roots of the two species of Oak above mentioned where it was regarded as growing saprophytically without forming typical mycorrhiza in the younger roots. The matted strands of mycelium or rhizomorphs were common in the soil about the trees, frequently spreading to potato fields in the neighbourhood, in which case the fungus behaved as a true parasite, attacking and destroying the potato tubers. Infection of the *Gastrodia* tuber by the rhizomorph of the fungus was effected by haustorial branches which penetrated the corky covering and formed localised areas of infection, the whole relation showing a close superficial resemblance with that of a *Cuscuta* plant and its host. The infected area of the tuber showed three distinct regions differing in the structure of the cells and the character of the intracellular mycelium. The hyphae in the outer tissues remained unaltered: in the middle region they attacked and consumed the protoplasm and nuclei, passed through a stage that simulated "clumping" in the root cells of Orchids and subsequently underwent complete autodigestion: in the inner tissues they were subjected to rapid digestion by the cells of the tuber. Remarkable cytological changes in the infected cells were described by Kusano. The cells of the inner region in particular showed signs of great metabolic activity, becoming filled with secondary food products. In the earlier stages of infection starch disappeared from all the infected cells, re-appearing in those of the inner region at the close of the period of active digestion.

The association between tuber and fungus was apparently casual and took place only occasionally. When it occurred, a full-grown offset was formed which flowered the following spring, otherwise small daughter tubers were produced which dwindled without flowering (Figs. 27, 28).

The relation in this species of *Gastrodia* is a very puzzling one. Subsequent to the formation of external cork, the tubers are unable to absorb liquids from the soil and apparently depend solely upon the chance of infection for a means of communication with the outside world. It is not clear that the fungus can derive even a temporary benefit from the association although this is evidently of a parasitic nature in the first instance. On the other hand, the *Gastrodia* plant evidently profits, since the ability to form flowers and fruit depends

directly upon invasion. It was suggested by Kusano that the Orchid plant has converted a parasitic attack by the rhizomorphs into a means whereby food materials are transferred from the Oaks or other plants to which the mycelium of the fungus is attached. If so, a remarkable case indeed of a vascular plant turning the tables upon one of the most destructive fungus parasites known!

There is as yet no information as to the behaviour of seeds of *Gastrodia* nor evidence of the existence of any relation with an endophyte of the *Rhizoctonia* type common to other Orchids. At present the case stands as an isolated and unique instance of a non-chlorophyllous vascular plant which depends upon a parasitic relation with a fungus for the completion of its reproductive cycle.

The physiology of another species of *Gastrodia*, *G. sesamoides*, has recently been studied by McLuckie (1924). This endemic Australian Orchid possesses a perennial rhizome with a few roots and colourless scale leaves. The flowering shoot develops from the apex of the rhizome and consists of a brown, slightly succulent axis up to eighteen inches in length bearing a few membranous scale-like leaves and a terminal inflorescence.

The rhizome shows both fungal and bacterial infection. The former is limited to isolated groups of superficial cells whence hyphae extend outwards into the soil; the coarse non-septate mycelium is intracellular in distribution but is not digested by the host cells. Bacterial infection is profuse, affecting not only the tissues of the rhizome but also those of the roots and flowering shoot. In artificial cultures the bacteria were found to assimilate free nitrogen at the rate of 7.8 mg. of nitrogen per 100 c.c. of culture solution in 15 days.

This species of *Gastrodia* had been previously recorded as a root parasite. The observations just recorded led McLuckie to class it as a saprophyte associated symbiotically with a mycorrhizal fungus and a nitrogen-fixing bacterium. It is believed that the Orchid is directly and indirectly dependent upon its endophytes for both carbonaceous and nitrogenous food-materials, and it is suggested by the author that the association is an obligate one. It is thought that the mycelium functions by absorbing water and mineral salts, and is likewise responsible for the intake of organic carbon compounds and possibly also of organic nitrogen compounds from the soil. Assuming the correctness of this interpretation, the bionomics of the species form an interesting contrast with those of *Gastrodia elata*.

Another uncommon non-chlorophyllous Orchid, *Dipodium punctatum*, has also been investigated by McLuckie (1922). This is a ter-

restrial plant occurring in the "shaded humus soils of the Australian Bush." The reddish-coloured flowering stems, bearing small scale leaves, arise from a few large succulent roots which penetrate the humus in various directions. Like *Monotropa*, the species had been described as a root parasite on neighbouring plants (Moore and Betsche, 1893). No evidence of parasitism was found by McLuckie, whose observations led to the view that in this case also the root fungus supplied its host not only with organic substances from the humus, but also with water and salts. Whence followed the logical conclusion that "in *Dipodium*, 'symbiotic saprophytism' has practically become a case of the higher plant being parasitic upon the endophyte."

The roots are remarkable in the possession of a sheath resembling the velamen of aerial roots, the thin-walled, living cells of which fit closely together and appear to constitute an "aqueous tissue." The mycorrhiza is of the familiar Orchid type with extensive intracellular infection of the cortical cells, followed by digestion and disappearance of the stainable products.

McLuckie's conclusions as to the real significance of root infection in this Orchid are supported by evidence of the usual kind—absence of root hairs and an assimilatory mechanism in the vascular partner, the known ability of the endophyte to utilise organic compounds of carbon and nitrogen, wholesale digestion of the intracellular mycelium—there is, indeed, a strong presumptive case for parasitism on the endophyte, not only in *Dipodium*, but in most of these so-called vascular "saprophytes." The pressing need at the moment is for *experimental* evidence bearing on the physiological relations between the symbionts, and for proof that the vascular plant alone is unable to utilise the nutritive substances locked up in humus. Such evidence can be obtained only by "pure culture" methods, which present exceptional difficulties in the case of non-chlorophyllous species, owing to the initial difficulty of securing seed germination.

Knudson (1922) began a fresh chapter in the story of the Orchids and their root fungi with his paper entitled, *Non-symbiotic germination of Orchid Seeds*, to which two others have since been added (Knudson, 1924, 1925). His interpretation of the observed facts differs from that of Bernard; following a review of the three papers just mentioned the nature of this disagreement will be discussed. In the first, he described the germination of seeds of *Laelia*, *Cattleya* and related forms in aseptic cultures by the addition of certain sugars,

or of plant extracts with traces of sugar, to the substrata. He recorded also the interesting new observation that inoculation of the seed cultures with a strain of *Bacillus radiculicola* isolated from Alfalfa, produced a beneficial effect upon germination and the development of chlorophyll. Two years later he reported that he had obtained practically 100 per cent. germination of seeds and the subsequent production of healthy seedlings of species of *Cattleya*, *Laelia*, *Epidendron*, *Cymbidium*, *Phalaenopsis*, *Dendrobium*, *Ophrys*, *Cypripedium* and *Odontoglossum*, i.e. that these non-symbiotic methods can be used with equal success for raising seedlings of the more refractory species. He found likewise, that when germination had advanced to a certain point, the seedlings became independent of an artificial supply of sugar, and could be transplanted to a suitable nutrient containing inorganic salts only, where they continued to make healthy growth quite independently of fungus infection (Fig. 26).

From these experiments Knudson deduced certain conclusions among which may be noted the following: (1) that the necessity for symbiotic fungus infection has not been proved; (2) that the germination of Orchid seeds is dependent on an outside source of organic matter, e.g. sugar, the effect of which is purely nutritional; (3) that young Orchid embryos, germinated aseptically without sugar, are unable to photosynthesise even if chlorophyll is present, such inability being presumably due to lack of some internal factor.

Knudson subsequently extended his researches and repeated and confirmed certain experimental observations made by Bernard. Thus, he isolated the endophytes from *Cattleya*, *Cypripedium*, and *Epipactis* and used the three strains—apparently regarded by him as identical in physiological action as in morphological character—for the successful germination of *Cattleya* seed. He made two observations of special interest, one, that the inoculation of seed cultures with certain other fungi, e.g. *Phytophthora* sp., produced comparable effects—“*Phytophthora* sp. is about as favourable to germination as the orchid fungus”—a fact already put on record by Burgeff for casual infection by *Penicillium*; the other, that successful non-symbiotic germination was obtained on a sterilised peat and *Sphagnum* mixture by the addition of a solution of inorganic salts of hydrogen-ion concentration equal to pH 4.6, the germination being equally rapid as when the fungus was supplied. No explanation whatever of this latter observation can at present be offered beyond the assumption put forward by the author himself; namely, that under the conditions described,

the embryos obtained the requisite soluble organic food material direct from the sterilised substratum. It must be noted that the seeds used were those of *Cattleya* which are known to reach a fairly advanced stage of development in aseptic cultures without inoculation.

In short, Knudson has confirmed the *fact* of symbiotic germination under experimental conditions but rejects Bernard's views as to its significance. Discussing this disagreement, Knudson stresses the importance of the nutritional aspect of the problem. In his view, the sugar supplied to germinating seeds acts as a food, and the beneficial result of inoculation by a suitable fungus in Bernard's experiments depended, not upon infection of the embryo tissues or upon any internal effect, but upon the production of sugar from organic material supplied in the medium with a corresponding rise in the concentration of the substratum. The effect produced in Knudson's cultures both by the specific endophytes and by certain foreign organisms is similarly explained. In addition to the production of sugar from starch, Knudson noted a marked rise in the hydrogen-ion concentration of inoculated cultures. For example, in one series, concentrations equal to pH values of 7.0, 6.3 and 6.0 were changed to pH values of 4.6, 4.8 and 4.0 respectively, indicating a decided increase in the acidity of the substratum. In the more acid media healthy seedlings were produced, in the others the embryos perished. Commenting on these facts he observes: "Here is evidence that the hydrogen-ion concentration is markedly increased by the fungus, and this must be due to organic acids produced by the fungus."

Knudson confirmed Bernard's and Burgeff's observations on the specificity of the endophytes but again offered a new interpretation of the observed facts. Noting that seeds of *Odontoglossum* were parasitised and killed by the fungal strains isolated from *Cattleya*, *Epipactis* and *Cypripedium*, he concluded that "these fungi are extremely pathogenic." But selective pathogenicity of this kind at once raises biological problems of profound interest, and it was to the analysis of such problems that Bernard devoted his last years. Had he lived longer, it can hardly be doubted that he would have turned once more to the practical applications of his discoveries, more especially to the improvement of methods for effecting non-symbiotic germination. He had already discovered and noted that this method was safer and easier than that of fungus infection, owing to the difficulty of maintaining suitable strains of the endophytes in artificial cultures.

Knudson's more important theoretical conclusions are indicated in the following passages: "The explanation of the failure of orchid seeds to germinate when provided with all the conditions that permit of the germination of most seeds, is to be found in the organic food relations. The seeds of orchids are lacking in food reserves. . . . Growth of the embryo will continue for a time at the expense of the reserve food, but ceases sooner or later. If the embryos are then supplied with sugar, growth will continue and germination occur. . . . If the embryos are carried over this critical period, then they are thereafter self-sustaining. The significant fact has been noted that the seeds of terrestrial orchids may germinate in nature under conditions where chlorophyll is entirely lacking. These embryos are purely saprophytic. The conclusion seems to be warranted that under natural conditions the orchid embryos are dependent for continued development on an appropriate supply of organic food, which must be absorbed from the material on which the seeds are germinating. Under natural conditions, this food is made available to the orchid embryo by the digestion of organic matter, which transforms the insoluble substances to soluble products. Some of these substances are absorbed by the embryos and are used in the metabolic process. Under natural conditions the orchid fungus may function in these digestive processes, but it would be pure assumption to conclude that no other micro-organisms are involved in this transformation. Sugars are undoubtedly formed, although the concentration would be low. It would seem that other substances are more effective."

The interest and practical value of Knudson's work is not to be denied. To the writer it seems unfortunate that he should have marshalled his experimental results in the form of an argument against the "so-called symbiotic theory of germination" attributed to Bernard and Burgeff. In the main, the new facts constitute an extension rather than a refutation of Bernard's work. It is true that the latter, who originally recorded the rise in concentration of the substratum brought about by fungus growth and also the practicability of non-symbiotic germination, was temporarily too pre-occupied with the mechanism of immunity and resistance in Orchids to carry further his observations on the parallel effects produced by infection on the one hand and an increased concentration of sugar *outside* the tissues on the other. It is equally true that the explanation put forward by Knudson, providing as it does an adequate and intelligible physical basis for the effect produced by the presence of the fungus, whether in the tissues of the embryo or in the sur-

rounding medium, does not cover all the known facts. It does not explain, for example, the invariable association of particular fungus strains with individual species of Orchid in nature, the toleration of mycelium in certain tissues, or the fungicidal action exerted by others.

Observations on the effects produced on germination in Bernard's experiments by the use of cultures of different ages led Knudson to conclude that "the most active fungus is the weakest pathogen." They led Bernard, with equal justice, to the view that the absence of obvious pathogenic symptoms in Orchids was due to an acquired resistance similar in kind to that displayed by the "carrier" in the realm of animal pathology. To him, the Orchid fungi were all pathogens. As with pathogenic organisms which attack animals and man, the degree of "virulence" exhibited would be expected to vary directly with the condition of the host and with the history of the attacking organism in pure culture. He was far from underrating the risks involved in the symbiotic method of germination under laboratory conditions. "For five years," he writes, "I have sown seeds of various species of Orchid in culture tubes each containing one hundred seeds, and have inoculated these cultures with *Rhizoctonias* extracted from the roots... On the whole, I have obtained some hundreds of plants, but I underestimate when I place the total number of seeds used in my experiments at 50,000" (Bernard, 1909 a).

That healthy seedlings of certain Orchid species can be grown for several years under experimental conditions without infection has been proved by Knudson and others. The problem which engaged Bernard's attention was not the production of such artificially "sheltered" plants, but the explanation of a condition found *invariably* in nature and its relation, if any, with the observed irregularities in seed germination.

In the meantime, any complete explanation of the association of Orchids with particular root fungi must take into account the facts just mentioned. For which reason the "further investigations on the food relationships of orchid embryos" promised by Knudson will be awaited with great interest.

Constantin and Magrou (1922) have subjected the views expressed by Knudson in his earlier paper to severe criticism. It has been pointed out by these authors, as in the present review, that the observation recording the fact that the effect of the fungus on germination could be replaced artificially by a supply of sugar or other nutrient is not a new one, and in itself provides no explanation either of the invariable presence of the endophytes in Orchid roots,

or of the specificity exhibited by the forms associated with particular Orchid species.

That root infection is practically invariable in Orchids has been amply demonstrated by Wahrlich and others. For further evidence on this matter attention is directed to the more recent observations by Constantin and Dufour (1900) on *Goodyera repens*. Examination of the rhizomes of an immense number of plants of this species by one of these observers, not only confirmed the fact of invariable fungus infection, but provided evidence of the presence of an identical strain of the endophyte, named by the authors *Rhizoctonia Goodyera repentis*, in every case examined.

The researches of Huber (1921) on *Liparis loeselii* are significant in the same connection.

Since the middle of the nineteenth century when they were described by Irmisch (1847, 1850, 1854, 1863), the European Orchids belonging to the small group Malaxoideae have been known to possess morphological peculiarities, e.g. the presence of aerial tubers resembling those of tropical species. In an account of these special features Goebel (1901) included a brief account of the remarkable distribution of mycelium in the vegetative organs. With this exception nothing was known of the mycorrhizal relations previous to Huber's work, a fact doubtless accounted for by the extreme rarity of the species.

Huber confirmed Goebel's observations and undertook an investigation into the history of infection in the individual plant of *Liparis loeselii* together with the isolation of the endophyte and its behaviour in pure culture. The facts elucidated in the course of this research are of interest and have a direct bearing on the existence or otherwise of an obligate symbiotic relation.

The isolation of the endophyte presented no difficulty. It proved to belong to the type, *Rhizoctonia repens* Bernard, or *Orcheomyces psychodis* Burgeff, and agreed with the forms previously described by the latter author in respect to nutrition. Two features of special interest may be noted in Huber's account; namely, the unusual distribution of fungus infection in the vegetative organs, and the artificial production and attempted cultivation of plants entirely free from fungus infection.

Liparis is a chlorophyllous species with no obvious peculiarities relating to nutrition. The rhizomes show invariable and profuse fungus infection of the cortical tissues, the mycelium in a majority of the infected cells eventually undergoing digestion in the usual

way. The roots and leaf-bases are also infected but the amount of mycelium present in these organs is relatively scanty. In the epidermis of the roots and leaf-bases, the endophyte forms chains of spores identical with those observed in pure cultures. Seed germination is replaced by a profuse development of adventitious buds on the aerial tubers. With regard to the course of infection, the mycelium present in the old rhizome does not spread into that of the succeeding year. Infection of the latter is accomplished indirectly through the roots which become invaded by growing into the cortical tissues of the old axis. In the initial stages, therefore, the bud from which will develop a new vegetative axis is entirely free from mycelium, and, if separated with due precautions from the older parts, can be further grown independently on a sterilised substratum.

By taking advantage of this, Huber was enabled to test the capacity of the mature plant for independent growth lacking fungus infection. Buds were removed in January and developed freely to a height of some centimetres on a sterilised substratum. Microscopic examination showed the plantlets to be entirely free from fungus mycelium, the cells of the cortex possessing small nuclei and an abundance of starch and thus differing markedly from those in normally infected plants. Subsequently, growth fell behind that of the uninfected controls; the infected plants became more susceptible to adverse external conditions, did not produce flowers, and by the middle of July all had succumbed. It was inferred by Huber that infection is an obligate condition for full development: "Die Unentbehrlichkeit des Pilzes auch für die erwachsene Pflanze ist damit erwiesen."

Apart from its bearing on the question of non-symbiotic germination, the condition described for *Liparis* shows many features of interest. It has been suggested by Huber that the symbiotic relation in the Malaxoideae is relatively simple, the endophyte showing little modification and the infected tissues being relatively primitive in respect to the differentiation of "Pilzwirhzellen" and "Verdauungszellen."

The lack of agreement between the conclusions of Bernard and Burgeff respecting the efficacy of old fungus cultures to effect germination has been investigated experimentally by J. Wolff (1923), who has expressed the opinion that Bernard did not sufficiently take into account the age and origin of Orchid seed used for experiments in estimating the effect of the age of fungus cultures upon germination. Wolff's experimental results indicate that the rate of

germination and the germination capacity of seeds of some species of Orchids decrease rapidly with age, those more than three months old failing to germinate at all. It was recorded subsequently (Wolff, 1925) that of seeds sown on a medium containing 2.5 per cent. glucose, some lost their germination capacity in 45 days, others in two to four months; if stored dry, *in vacuo*, they retained their vitality for longer periods. Knudson (1922) also reached the conclusion that Orchid seeds should be germinated as soon as possible after collection.

Wolff (1924) has also investigated the injurious effects sometimes observed when Orchid seeds are germinated in highly concentrated media in contact with mycelium of the appropriate endophyte. By changing the composition of the medium, it was found possible to produce seedlings of *Cattleya* resistant to the injurious effects of a mycelium pathogenic to them under other conditions. For example, seeds of *Cattleya* germinated asymbiotically on a medium containing glucose were able to resist attack by a mycelium which killed seedlings germinated directly in its presence.

The use of "pure-culture" methods for the raising of Orchids from seed has been adopted with success by practical growers both in Great Britain and other countries. An admirable account of the remarkable work accomplished by the late Mr Charlesworth in his own nurseries has been contributed by Ramsbottom (1922), who was associated with the work (Fig. 25). Analogous results have been obtained in Belgium and in Germany. To the success of the method in the hands of French growers witness has been borne by Bultel (1920, 1925) and Constantin and Magrou (1922).

To an account of his own successful experiments on the raising of Orchids, M. Bultel added: "La culture que nous pratiquons de préférence est la culture aseptique en tubes sur milieux gélosés ou autres ensemencés du Champignon endophyte; ce procédé présente sur tous les autres le grand avantage d'une *réussite assurée*, et aussi celui de ne demander aucun soin depuis le jour de semis jusqu'au moment du repiquage des jeunes plantules, soit plusieurs mois après."

More recently the same observer (Bultel, 1925) has reviewed the older work on the symbiotic relations in Orchids, and discussed the degeneration and regeneration of the endophytes in artificial cultures. He has described, also, his own methods for isolating the root fungi and for raising seedlings both by symbiotic and asymbiotic methods. The contention that Orchid seedlings raised asymbiotically grow

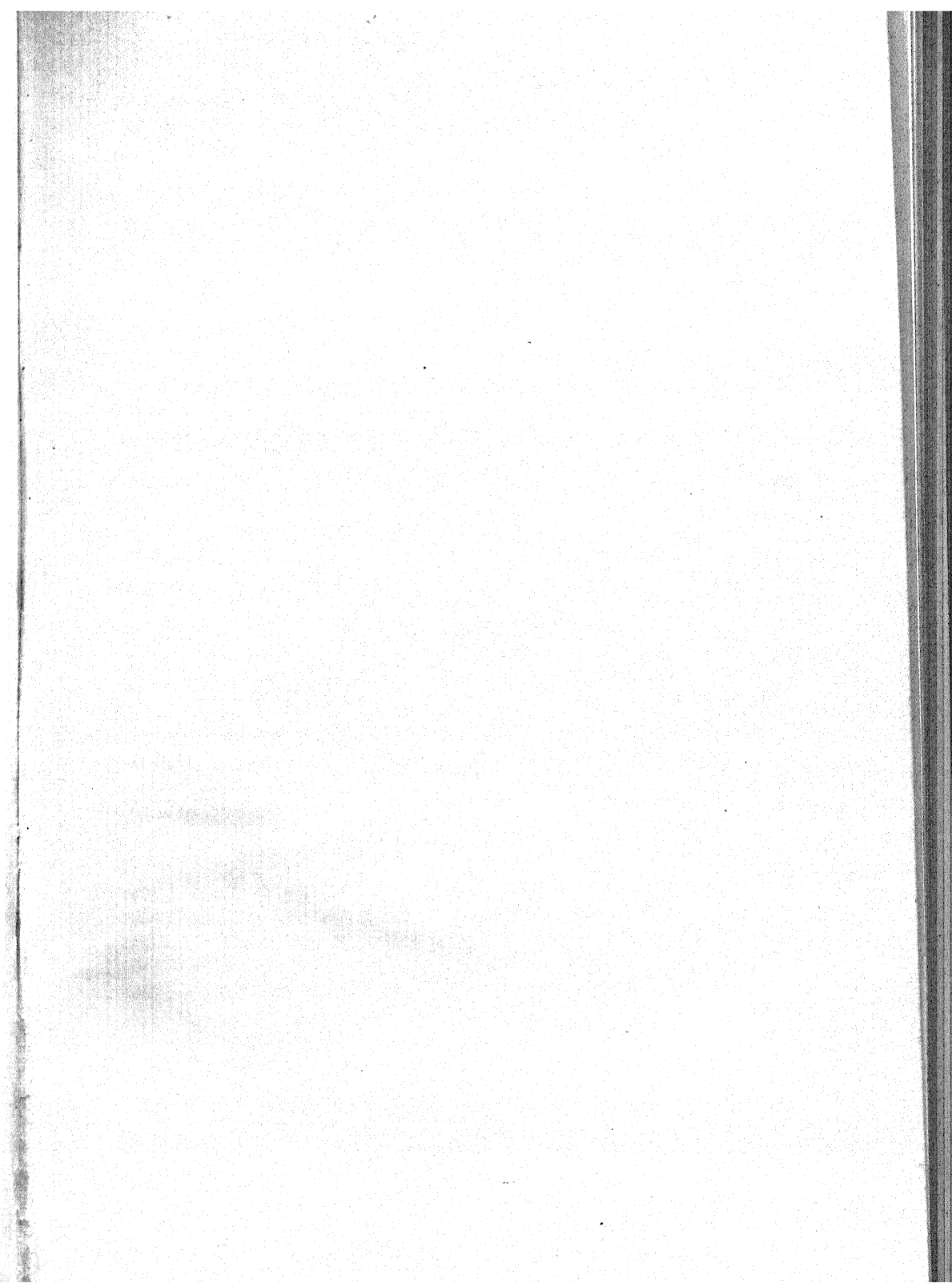
into abnormal plants incapable of flowering is not supported by his experiments. On the contrary, he has placed on record the case of two hybrid Orchids, raised without infection, that produced flowers in the normal way.

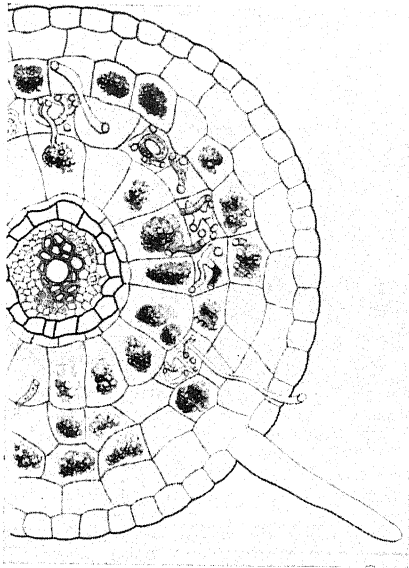
To the professional horticulturalist, the symbiotic method of germination still presents obvious difficulties. The isolation of the specific endophyte is a delicate operation, as is the maintenance of strains in a suitable condition for promoting germination. Moreover, the sterilising of culture vessels and media involves the use of special apparatus not ordinarily needed in horticultural practice. As pointed out by Bernard himself, the use of non-symbiotic methods would remove several of these practical difficulties and greatly expedite the raising and cultivation of Orchids under glass. More especially would this be so, if the claims made recently by Knudson and others in respect to the growth of fungus-free plants stand the test of further experiments and can be shown to apply to horticultural conditions. In the meantime, practical growers in Great Britain and elsewhere are experimenting with non-symbiotic methods, and in certain cases have reported very favourably.

A remarkable collection of seedling cultures raised asymbiotically on artificial media containing sugar and other organic substances was exhibited recently in London, the tubes in every case being crowded with healthy seedlings (Fig. 24). The cultures included seedlings of species of the more refractory genera, e.g. *Odontoglossum*. The exact constitution of the media used was not stated, but the worker responsible for these cultures has placed on record certain interesting facts relating to his own experiments. He has confirmed Knudson's observation that the reaction of the substratum exerts an important effect upon germination.

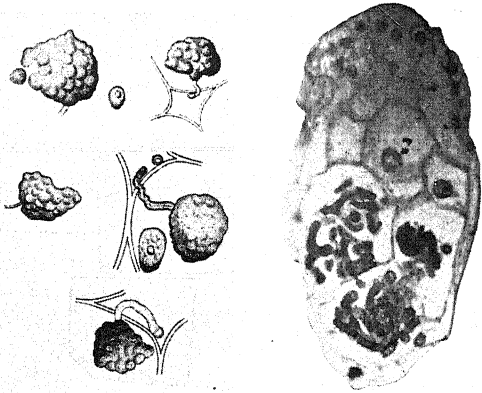
For seeds of *Odontoglossum*, the best results were obtained with pH values ranging from 6.5 to 6.8. Special attention has been paid to this genus, and it was found that a medium which promoted germination of seeds of one species of *Odontoglossum* was unsuited for those of another and that it was necessary to carefully adjust the constitution of the media to the needs of individual species. The exact significance of these facts is not at present clear, but they are of interest in view of the difficulties experienced in obtaining successful germination of seeds of *Odontoglossum* by the ordinary horticultural methods (Clement, 1924).

No aspect of the physiology of the symbiotic relation in mycorrhiza is of greater interest than that relating to the possibility of



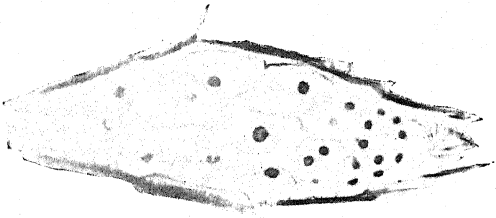


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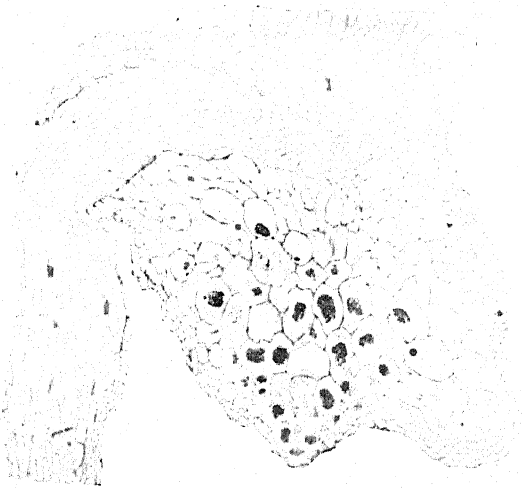
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nitrogen fixation by the endophytes. A preliminary account of new researches on the root fungus of *Neottia* by H. Wolff (1925) has reopened this question in relation to the Orchid endophytes (see p. 94).

Wolff reports that the fixation of atmospheric nitrogen by the endophyte of *Neottia* has been quantitatively proved in pure culture, the fungus being able likewise to use organic compounds, e.g. glycol, and also ammonium salts as sources of nitrogen. A large number of compounds were utilised as sources of carbon, e.g. glucosides (tannin), polysaccharides, hexoses, pentoses and pentosans. Fuchs and Zeigenspeck (1924) had previously shown that the fungus of *Neottia* could utilise similar compounds in nature, and had demonstrated the presence of the appropriate enzymes in infected root cells. It is surmised by Wolff that these enzymes are produced by the mycelium and not by the cells of the host. His present researches seek to demonstrate that compounds of carbon and nitrogen formed by the fungus eventually fall to the share of the *Neottia* plant. A further report will be awaited with interest.

It is worthy of note that the small family Burmanniaceae, containing a number of remarkable non-chlorophyllous species of very reduced habit, is closely related to Orchidaceae. (See Plate I.) Like the Orchids, members of the former group produce seeds with scanty reserves and also develop typical mycorrhiza in the root system.

There are at present no experimental data concerning germination or the biology of infection and its relation with nutrition, but it would not be surprising to learn that in this group, as in Orchids, there has been evolved an association more intimate than that found in mycorrhizal plants generally.

EXPLANATION OF PLATES II AND III

PLATE II

- Fig. 16. "Sporangioles" of different plants as figured by Janse. (From Janse, *Ann. Jardin Buitenzorg*. 14, 1896-97.)
- Fig. 17. *Polygala amara*. Transverse section of root with mycorrhiza. (From Marcuse, 1902.)
- Fig. 18. *Odontoglossum* sp.: longitudinal section of seed. (From Ramsbottom, *Brit. Mycol. Soc. Trans.* 1922.)
- Fig. 19. *Odontoglossum* sp.: longitudinal section of seed, nine days from sowing, showing infection of the larger cells at the suspensor end and the formation of "pelotons." (From Ramsbottom, *Brit. Mycol. Soc. Trans.* 1922.)

Fig. 20. *Odontoglossum* sp.: section of protocorms. The stem apex and first two leaves can be seen at the upper end. In many of the infected cells, the mycelium is already digested. (From Ramsbottom, *Brit. Mycol. Soc. Journal*, 1922.)

Fig. 21. *Odontoglossum* sp.: longitudinal section of young seedling after formation of first root. The root is uninfected; the dark patches in the root-cells are raphides. (From Ramsbottom, *Brit. Mycol. Soc. Journal*, 1922.)

PLATE III

Fig. 22. *Platanthera chlorantha*. Tangential section of root showing "Pilzwirthezellen" and cells containing raphides. (From Burgeff, 1909.)

Fig. 23. *Platanthera chlorantha*. Radial section of root showing "Verdauungszellen." (From Burgeff, 1909.)

Fig. 24. Asymbiotic germination of Orchids. Seedlings of *Odontoglossum* sp. in organic nutrient 18 weeks from sowing. About natural size.

Fig. 25. Symbiotic germination of Orchids in horticultural practice. Seedlings of *Odontoglossum* sp. in sterilized compost infected by the endophyte, four months from sowing. $\times \frac{1}{3}$.

Fig. 26. Asymbiotic germination of Orchids. Seedlings of *Cattleya* and *Laelio-Cattleya* two years old on full nutrient without sugar. (From Knudson, *Bot. Ganz.* 1924.)

Fig. 27. *Gastrodia elata*: (1) Adult tuber with flower shoot.

(2) Inflorescence axis.

(3) Rhizomorphs of *Armillaria mellea* (*Rhizomorpha subterranea*) from soil about tubers of *Gastrodia*.

(4) Living stock of *Quercus serrata* with rhizomorphs of *Armillaria mellea*.

(5) Fruit bodies of *Armillaria mellea* from dead stump of Oak in neighbourhood of *Gastrodia*.

Fig. 28. (6, 7) Development of young tubers without association with fungal symbiont: (a) at end of May; (b) at beginning of August; (c) at beginning of April in the following year.

(8) Rhizome-like tuber of *Gastrodia*.

(9-12) Small tubers making organic connection with rhizomorph strands (collected end of May).

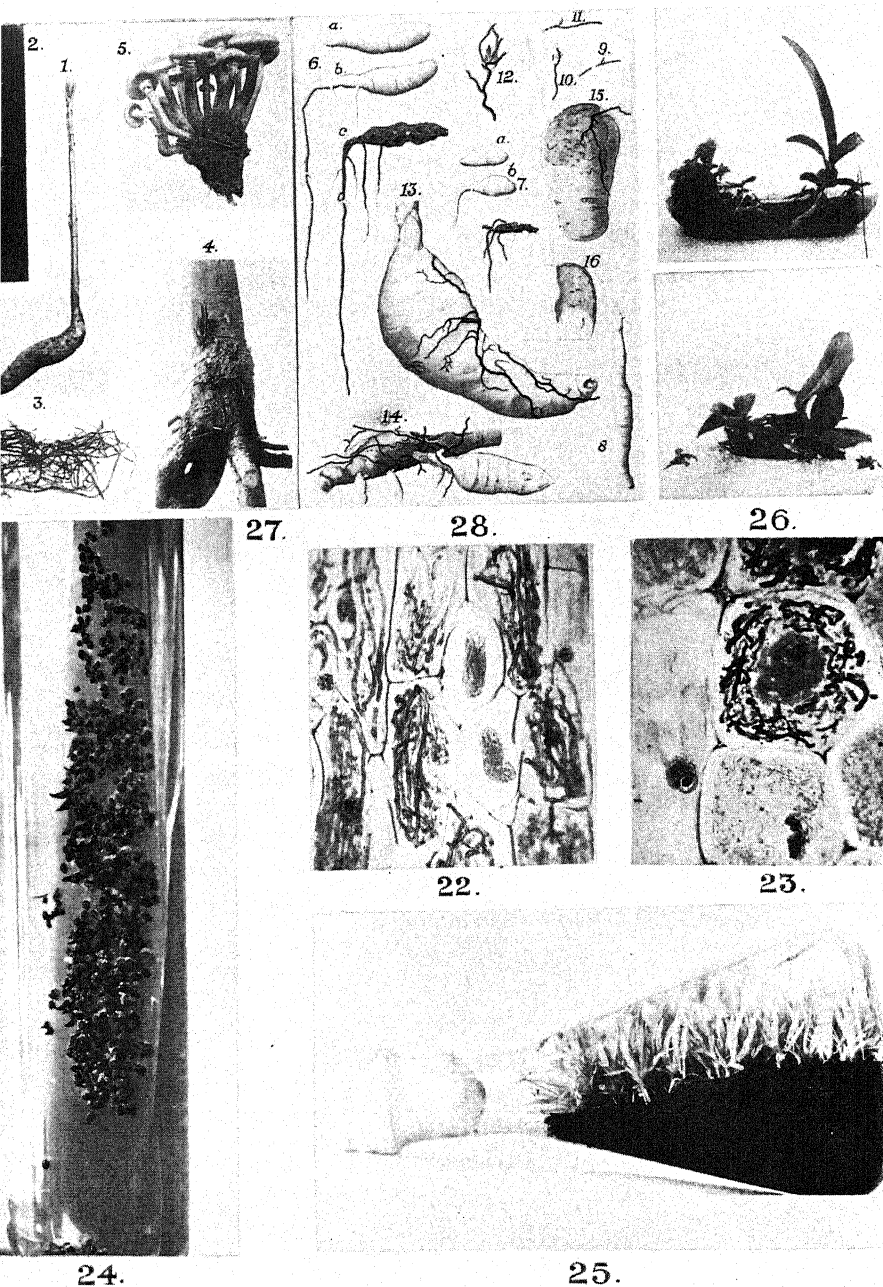
(13) Adult flowering tuber occasionally attacked by rhizomorphs.

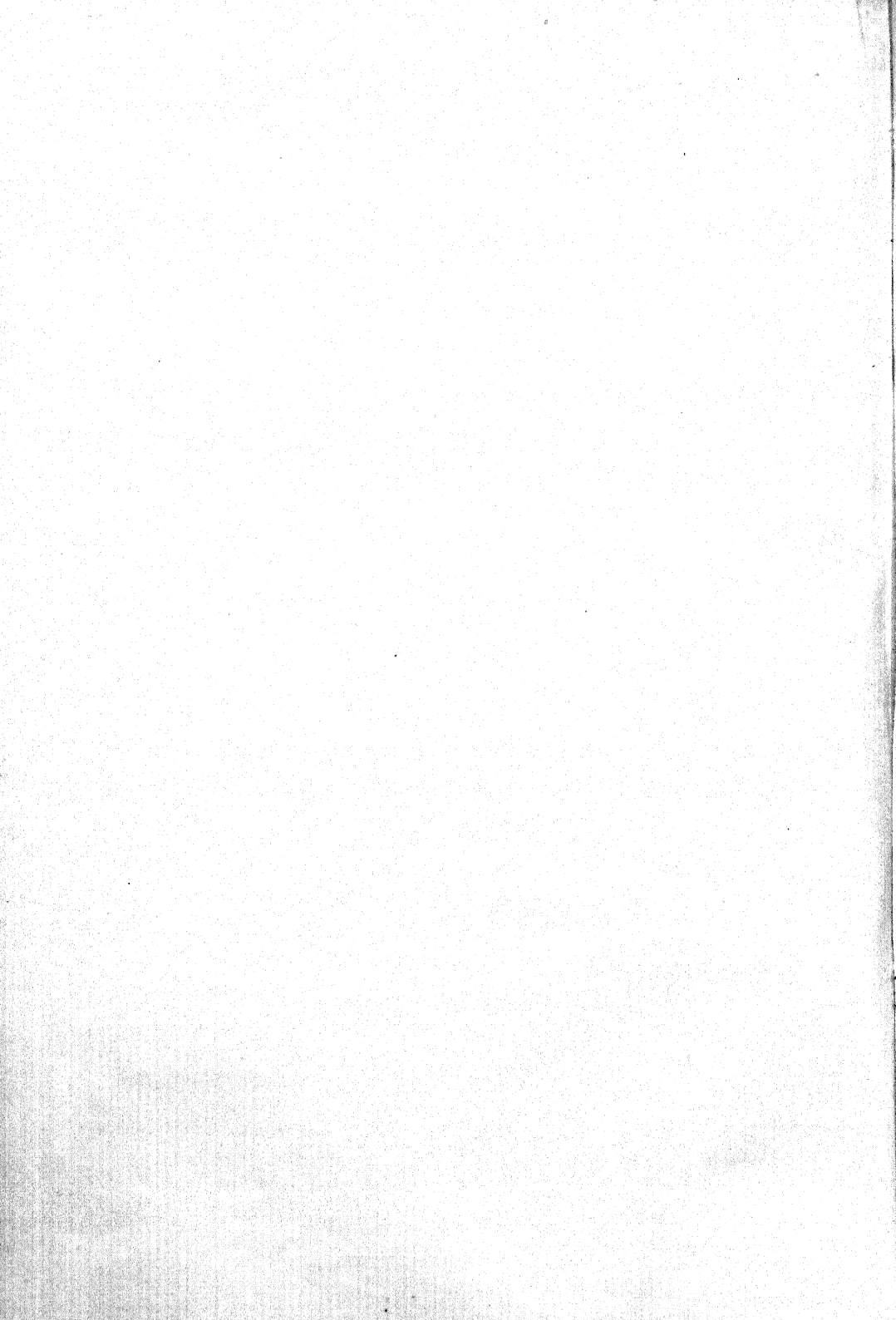
(14) Mycorrhiza formation in tuber laid under Oak tree in May, showing vigorous development and formation of offsets (observed in September).

(15) Potato tuber attacked parasitically by rhizomorph of *Armillaria mellea*, showing discoloration and collapse of affected tissues.

(16) Section through affected tissue of same showing distribution of strands as "*Rhizomorpha subcorticalis*." (All from Kusano, *Journ. Coll. of Agric. Tokio*, 1911.)

(To be continued)





THE KATHAROMETER AS AN INSTRUMENT FOR MEASURING THE OUTPUT AND INTAKE OF CARBON DIOXIDE BY LEAVES

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(With 4 figures in the text)

IT would scarcely be an exaggeration to say that physical analysis can generally be carried out with greater accuracy and ease than chemical analysis. But when the problem is essentially a chemical one and the physical method is employed, it is of course necessary to be able first to state the physical result in chemical terms, and be assured, beyond all possibility of doubt, of the exact relationship between the two.

Every gas has its own thermal conductivity, and this property has been utilised by G. A. Shakespear and by H. A. Daynes^(1,2) for the quantitative analysis of various gases and has been applied with marked success by The Cambridge Instrument Company, Ltd., to the analysis of carbon dioxide (CO_2) in flue gases. The commercial instrument for this purpose, known as the Katharometer, may be used not only for measuring differences of 1 per cent. of CO_2 within a range of 0 to 20 per cent., but, with suitable modifications, is also capable of detecting far smaller variations. It appears eminently adapted for measuring the respiration and photosynthesis of a single leaf, and the results obtained by the writer indicate in his opinion that the method may be of general utility in the study of certain problems of plant physiology. The following details of the experimental arrangements and of some results may therefore be of interest.

A current of 120 milliamps. flows through a Wheatstone bridge (Fig. 1). The two arms, *A*, *B*, are each of a constant resistance, approx. 10 ohms, and are made of manganin. The other two arms, *C*, *D*, each consists of a fine spiral of platinum, of approx. 10 ohms resistance, whose temperature is raised by passage of the current. The resistance of *C* (communicating with the control chamber) remains constant during any particular experiment. The resistance of the fourth arm, *D*, varies. Now its resistance will be a function

of its temperature, and this in turn will depend upon (a) the magnitude of the warming current and (b) the thermal conductivity of the air surrounding the spiral. The warming current is carefully regulated to 120 milliamps. by means of a variable resistance and milliammeter (not shown in the figure). With a 4-volt accumulator of 33 ampère-hours in good condition, the current does not as a rule vary appreciably during experiments lasting up to 15 hours, but can, if necessary, be readjusted.

The thermal conductivity of the air surrounding the platinum spirals depends upon its composition. Thus if the composition of the air in the control chamber remains constant, any change in the composition of the air in the experimental chamber will be indicated by a proportionate change in the electrical current through the gal-

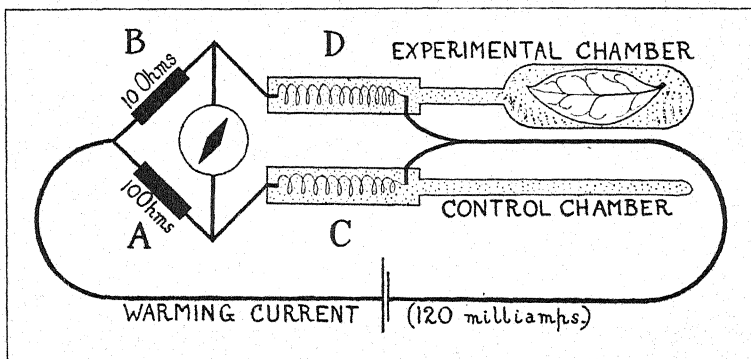


Fig. 1. The circuit.

vanometer. The gases which concern us are CO_2 , oxygen and water vapour. Increase of CO_2 causes a *decrease* of thermal conductivity and, according to Daynes, the relation between the two is *almost linear* over a large range. Measurements made with the instrument now described have been such as to confirm this almost linear relationship between approx. 0.15 and 0.6 per cent. CO_2 . It is hoped to examine the relationship outside this range in the near future.

Substitution of oxygen in nitrogen causes an *increase* of thermal conductivity and here also the linear relation holds good, but Daynes states that the effect of O_2 upon thermal conductivity is only about one-tenth that of CO_2 . Thus with a leaf whose respiratory quotient, $\frac{\text{CO}_2 \text{ exhaled}}{\text{O}_2 \text{ absorbed}} = 1$, the galvanometric readings will be 10 per cent. higher than they would be if caused by a change of CO_2 -concentration alone. But it is important to note that, for the particular

biological purpose to which the Katharometer is here applied, it is most convenient to calibrate the instrument by the use of air from which a volume of O_2 has been removed equal to each volume of CO_2 added. In this way correction for oxygen will be made automatically, *provided* that the respiratory quotient or the (apparent) assimilatory quotient, $\frac{O_2 \text{ exhaled}}{CO_2 \text{ absorbed}}$, of the experimental leaf are respectively = 1.

In practice the calibrations have been made with diluted expired air from a normal man, whose respiratory quotient would not be less than 0.8. Assuming this figure, the rates of assimilation and respiration of leaves, as given in the table below, are too high by 2.3 per cent.

It would be desirable to calibrate the instrument by means of CO_2 produced by combustion of pure carbon or by the respiration of some plant or animal tissue whose respiratory quotient is known to = 1.

A source of error inherent in the method is the variation from unity of the respiratory and assimilatory quotients of the experimental leaf. But that such variations are in general very slight for mesophytic leaves has been shown by a number of investigators, e.g. Maquenne and Demoussy(4). Kostytschew(3) however has found that with short periods of illumination the (apparent) assimilatory quotient undergoes marked deviations. As an extreme instance *Betula verrucosa* may be quoted. This leaf during a 3-minute exposure to sunlight gave an $\frac{O_2}{CO_2}$ ratio of 0.4. It may be calculated that if the leaves dealt with in the present note were behaving constantly in such a manner, the value for rates of assimilation given in the table would be too low by 5.8 per cent. However it seems most unlikely that in practice the error would approach so high a value, for the duration of illumination in my experiments has rarely been less than 10 minutes, usually 20 or 30 minutes, and Kostytschew has found that the longer the illumination, the closer does the assimilatory quotient approach to unity. Similar considerations would apply with regard to any variations of the respiratory quotient.

Bearing in mind however the above source of error, the results obtained, examples of which are given in the table below, indicate that O_2 determinations are scarcely required in the particular problems for which the instrument is here utilised. As a preliminary calibration for the results reported in this note, a galvanometric

deflection of 1 cm. is taken as equivalent to a change of CO_2 -concentration of 0.06 per cent.

It may be mentioned here that Mr W. K. Slater, of University College, London, whose apparatus I recently had the pleasure of seeing, has applied the Katharometer to the measurement of the respiration of extremely small pieces of animal tissue, and has developed a volumetric method which, when used in conjunction with the Katharometer, should permit of changes in O_2 as well as CO_2 being calculated. Mr Slater proposes to describe this method in the *Journal of Scientific Instruments*, and it is to be hoped that it will dispel any lingering doubt as to the accuracy of the Katharometer as an instrument for the study of respiration, and even of photo-synthesis.

Change of air pressure within the experimental chamber will, if sufficiently great, cause a marked galvanometric deflection. Such a change of pressure might be caused by an alteration of temperature or by a deviation from unity of the respiratory or assimilatory quotients. The null effect of temperature is shown by the control experiment with black paper described below. The effect of the second factor was tested as follows. The air in the experimental chamber was subjected to changes of pressure such as to diminish or increase its volume by a known fraction. It was found that a change of volume of 1 per cent. produced a momentary galvanometric deflection which almost immediately subsided, while even with a change of 4 per cent. the deflections were not of much account.

Picture therefore a leaf with an $\frac{\text{O}_2}{\text{CO}_2}$ ratio of, say, 0.4 and that a change of 1 per cent. of CO_2 -concentration has taken place in the experimental chamber: the change of air volume will = 0.6 per cent. Thus even assuming an extreme value for the assimilatory or respiratory quotients, the changes of air pressure in the experimental chamber will not cause any significant error.

Water vapour need not concern us, since both the experimental and control chambers are kept saturated with moisture by the presence of a little water. In this connection also it may be remarked that I have not been able to detect any difference between the action of air which had been bubbled through water, and air which had been passed through dehydrating reagents.

In concluding this brief account of possible errors in the method, it must be added that the Katharometer becomes less sensitive by about 1 per cent. for every rise of 4°C . (Daynes). Variations of room temperature will therefore not cause any significant effect.

In Fig. 2 are shown those parts of the apparatus which are of special interest. As will be seen from the description which follows, the greatest precautions are taken to eliminate differences of temperature between the two chambers of the Katharometer. A rise of temperature acts in the same way as an increase of CO_2 -concentration.

The cells containing the platinum spirals, also the manganin wires, are enclosed in a solid brass case. This is surrounded by

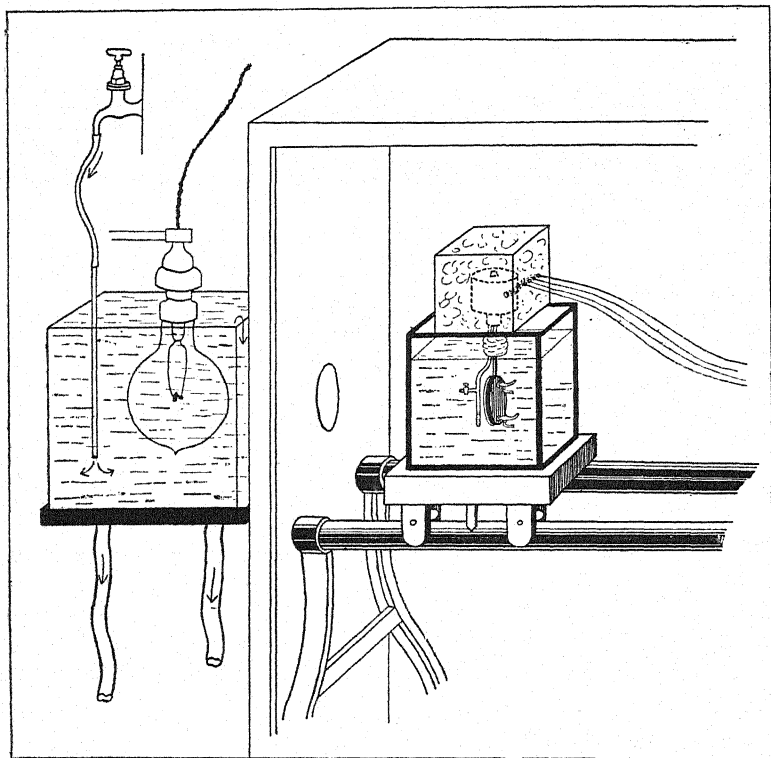


Fig. 2. Arrangement of apparatus for the study of photosynthesis under various intensities of illumination.

cotton-wool packed in a wooden box, so as to guard against local alterations of temperature. The experimental and control chambers, communicating with their respective cells in the Katharometer, are kept immersed in a glass tank of water in order to preserve them at a common temperature. The portions of the chambers situated between the surface of the water and the wooden box are surrounded with cotton-wool for the same reason.

The experimental chamber is adapted to the shape of the particular leaf (in the figure it is circular). Its volume should be as small as possible relatively to the size of the leaf, in order that changes of CO_2 -concentration may occur with the maximum possible rapidity, thus facilitating experiments. The control chamber may be of the same shape as the experimental chamber, but control experiments have shown that this is not essential. In Fig. 2 the control chamber is merely a glass tube of convenient shape. Both chambers are provided with side taps, which are kept open while the chambers are being affixed to the Katharometer, after which the taps are closed. If this precaution is omitted, a marked galvanometric deflection may be brought about through unequal pressures in the two chambers.

The source of light is a 1000 c.p. "Pointolite" lamp, immersed in a glass vessel through which flows a rapid stream of water, escaping down that side of the vessel which faces the Katharometer. The Katharometer, together with its tank, is mounted on a large optical bench along which it can be moved so as to obtain various intensities of illumination. The greatest intensity made use of has been that given at 21 cm. from the lamp (intensity = 32), the least intensity (= 1) that given at 120 cm. The optical bench, etc., are enclosed in a dark chamber, at one end of which is an aperture, provided with a shutter to admit or cut off the light.

Four wires lead from the Katharometer to the source of the warming current and also to the recording galvanometer which is situated in a separate dark-room. The galvanometer, which is of the moving-coil type, has a resistance of 21 ohms, and with a current of 1 micro-ampère gives a deflection of 125 mm. at a distance of 50 cm. Its deflections are recorded photographically upon a strip of bromide paper, borne on a revolving drum and travelling vertically past a horizontal slit¹.

Careful control experiments have been performed to ensure that the galvanometric records are not influenced by the differences of temperature which must arise between experimental and control chambers during exposure to the action of light. Only after such negative records have been successfully obtained can the Katharometer be utilised for the study of photosynthesis.

An example is shown in Fig. 3. Instead of a leaf, a circular piece of dull black paper was placed in the experimental chamber. Since

¹ For details see Waller, J. C., "Plant Electricity, I," *Ann. Bot.* vol. 39, pp. 515-538 (1924).

black paper absorbs more light than a green leaf, a negative test with black paper is more convincing than a test with, e.g. a boiled leaf, though negative tests with the latter have also been obtained. It will be seen from the record that no appreciable galvanometric deflection occurs during the 35 minutes' illumination at full intensity.

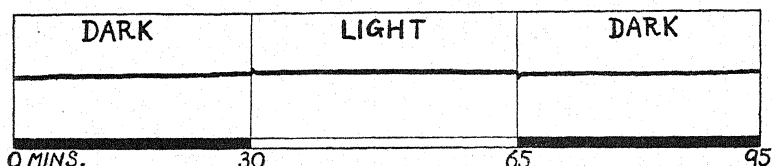


Fig. 3. Galvanometric record of a control experiment, showing that the effect of temperature variation between the experimental and control chambers has been eliminated. A piece of dull black paper is used in place of a leaf. Exposure for 35 minutes at full light intensity causes no significant deflection.

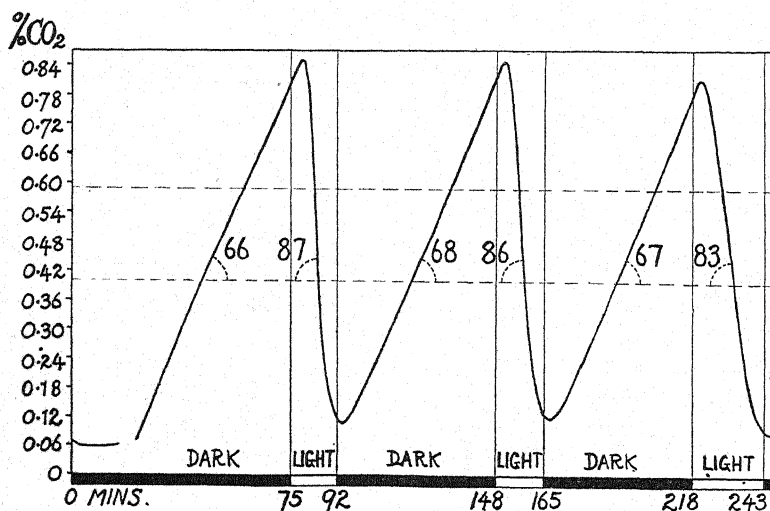


Fig. 4. Fern leaf. Galvanometric record of output and intake of CO_2 . Between the broken horizontal lines the changes of CO_2 -concentration are respectively of constant velocity and are proportional to the tangents of the angles which are marked in degrees. The initial portion of the tracing is taken with laboratory air in both experimental and control chambers.

There is a slight "nick" at the beginning and at the end of illumination, probably caused by the turning on and off of the electric-light current.

Fig. 4 shows an example of the type of record obtained with leaves. Upward motion of the tracing indicates output of CO_2 from

the leaf (respiration), downward motion intake of CO_2 (photosynthesis). The angle of the tracing depends on (a) the speed of the drum and (b) the rate of change of CO_2 -concentration. The speed of the drum is measured during the course of each experiment and the slight variations which occur are carefully scrutinised while working out results. A speed of about 1 cm. per 10 minutes has generally been found the most convenient for my purpose, but if greater accuracy is required, the speed has only to be increased. Assuming a uniform speed, the rate of change of CO_2 -concentration is proportional to the *slope* of the tracing (i.e. the tangent of the angle included by the tracing and the direction in which the record travels). The *uniform velocity* of CO_2 -production—and consumption—is shown by the *straightness* of the upward and downward arms of the tracing. This enables one easily to measure the angles to an accuracy of 1° . A diminution in the rate of CO_2 -consumption is seen towards the end of each illumination: this must be due to the diminished supply of CO_2 to the leaf, since it may be avoided by using a higher concentration of CO_2 to start with. An instrumental lag is also shown during the initial stages of light and of darkness respectively: this may be explained by the time required to establish a uniform rate of diffusion after the direction of the CO_2 -current between the leaf and its platinum spiral has been reversed.

It may be noted that with prolonged illumination, CO_2 -consumption may even come to a standstill, presumably owing to all the CO_2 having been used up by the leaf. Furthermore it has been frequently observed that subsequently to a period of rapid photosynthesis, there occurs an acceleration of respiration which gradually slows down. This is in accordance with the results of F. F. Blackman. It may be an after-effect of photosynthesis, but may be partly caused by a rise of temperature brought about through strong illumination.

To obtain comparative results with different leaves, the following data are required: the angle of the tracing, volume of the experimental chamber, dimensions of the leaf, speed of the drum, and value of galvanometric deflection in terms of change of CO_2 -concentration. Three examples are given below, the results from the record in Fig. 4 being at the beginning of the table.

To estimate "real" assimilation the rate of apparent assimilation is added to the rate of respiration in the right-hand column. This column gives the rate of respiration subsequent to each illumination.

If rates of assimilation are plotted against light intensities, some interesting curves will appear—whether to complicate or simplify the question of “limiting factors,” remains to be discovered.

TABLE
Assimilation and Respiration of Leaves

PLANT	RECORD NO.	DRUM SPEED MINS. PER 1 CM.	VOLUME AIR IN EXPERIMENTAL CHAMBER OF EACH C.C.	APPROX. PER CENT CO ₂ AT BEGINNING OF EACH ILLUMINATION	AREA OF LEAF, SQ. CM.	TEMP. °C.	LIGHT INTENSITY	APPARENT ASSIMILATION		RESPIRATION	
								ANGLE°	CO ₂ *	ANGLE°	CO ₂ *
<i>Pteris cretica</i> (August)	417	10.28	19.7	0.8	19.06	20.2	—	—	—	66	1.45
						20.3	32	87	13.15	68	1.60
						21.1	16	86	10.08	67	1.52
	418	10.29				21.5	8	83	5.75	67	1.52
						22.4	4	73	2.30	67	1.52
						22.5	2	41.5	0.62	65	1.38
						22.6	32	87	13.44	64	1.32
	419	9.93				22.7	32	86	10.43	64	1.37
						22.5	2	36	0.53	63	1.31
<i>Tropaeolum majus</i> (August)	415	10.11	81.2	—	68	—	—	—	—	63	1.47
						19.6	32	87.5	17.14	69	1.94
						20.1	16	87.5	17.14	72	2.30
						20.1	8	87.5	17.14	70	2.06
						20.2	4	84	7.11	69	1.94
						20.2	2	72.5	2.37	72	2.30
						—	32	88	21.43	71	2.17
						19.9	15	55	1.07	70	2.06
						19.8	32	88	21.43	—	—
<i>Brassica oleracea</i> (October)	429	4.85	14.2	1.3	11.66	20.8	—	—	—	33.2	1.05
						21.6	1	11.6	0.33	27.5	0.84
						21.8	2	47.8	1.77	31	0.97
						21.6	4	73.1	5.30	32.6	1.03
						21.2	8	79	8.28	34.6	1.11
						20.9	16	82	11.46	37	1.21
						20.6	32	82.4	12.06	—	—

* Mg. per hour per 100 sq. cm. leaf.

In conclusion I wish to thank Professor J. S. Macdonald for his unfailing help and for the stimulating interest which he has brought to bear upon this work: also Dr H. A. Daynes for helpful suggestions and answers to various enquiries, and for most kindly criticising this note previously to publication.

November, 1925.

Postscript added March, 1926. After writing the above paper I had the privilege of becoming acquainted with the work of Professor Noyons(5) at Louvain. His apparatus was designed independently of other investigators specially for the measurement of animal respiration.

The method has also been developed at the Bureau of Standards of the U.S.A.(6). The paper referred to includes an interesting account of the history of the method.

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THE CONTROL OF ATMOSPHERIC HUMIDITY IN A CLOSED SYSTEM

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(With 4 figures in the text)

IN experimental plant physiology the problem of the control of atmospheric humidity is one of considerable difficulty. The apparatus described in this paper has been designed to meet this difficulty.

Atmospheric humidity may be expressed in two ways, it may be stated in terms of "relative humidity," that is the percentage saturation of the atmosphere, or in terms of the "saturation deficit," this being the difference in vapour pressure between that of saturated aqueous vapour and that of the water vapour actually present in the air, both being measured at the same temperature. A brief consideration of the subject will show that these two concepts "relative humidity" and "saturation deficit" are widely different.

In, for example, the case of a somewhat moist atmosphere such as air which is 75 per cent. saturated at 15° C., the humidity may be expressed by the statement that the pressure of saturated aqueous vapour at 15° C. being equivalent to 12.73 mm. of mercury, the pressure of aqueous vapour present in the air under consideration is equivalent to 9.56 mm. of mercury, i.e. that 75 per cent. of the possible water is present; such an atmosphere is said to have a relative humidity of 75 per cent. at 15° C. The saturation deficit, on the other hand, is not expressed as a percentage but is given by the absolute difference between the two vapour pressures, being equivalent in this case to $12.73 - 9.56$, i.e. 3.17 mm. mercury.

An atmosphere 75 per cent. saturated at 25° C. would clearly have a very different saturation deficit from that of an atmosphere 75 per cent. saturated at 15° C., because at 25° C. the pressure of saturated aqueous vapour equals 23.55 mm., and the pressure of water vapour present must therefore be equal to 75 per cent. of this or 17.63 mm., giving a saturation deficit of 5.92 mm., so that although we have the same relative humidity in each case, the

saturation deficit, and hence the evaporating power, is very much greater at the higher temperature.

The relations between certain saturation deficits, temperatures, and relative humidities are shown graphically in Fig. 1.

In experiments on the plant it is, therefore, rather the saturation deficit than the relative humidity that should be maintained at a constant value, and attempts have been made in various ways to attain this end. One method is so to circulate the air in the system

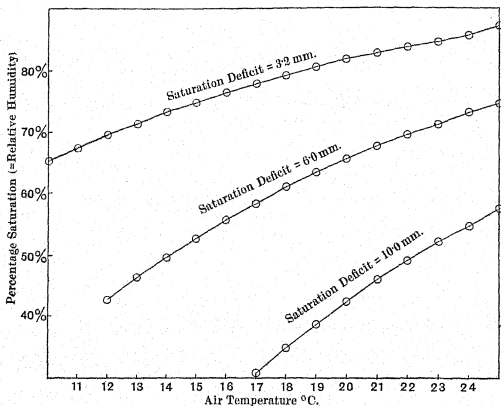


Fig. 1.

$$\text{Relative Humidity} = \frac{(x - y) 100}{x}$$

where x = pressure of saturated aqueous vapour at air temperature,
 y = saturation deficit.

containing the plant that this air passes through a solution of calcium chloride having the required vapour pressure. This method is open to several objections, among these being the fact that if any considerable amount of water is given up to or removed from the air a change in the concentration of the calcium chloride solution must result, thus changing the vapour pressure. Another objection is that if the air be circulated at all fast there is risk of minute globules of the fluid being carried over in spite of scrubbing devices.

Another and in many respects more satisfactory method is to saturate the circulating air with water at or above the temperature of the growing chamber, and then to pass it through a condenser which is immersed in a bath maintained at a temperature below that of the growing chamber, the temperature of the bath being such that saturation at this temperature gives the required saturation deficit when the temperature of the circulating air again rises to that of the growing chamber. The only important objection to this method of humidity control is that any change in the temperature of the growing chamber or of the condenser involves a change in the saturation deficit, and this difficulty cannot be overcome by maintaining the condenser at a temperature which differs by a fixed amount from that of the growing chamber, owing to the fact that, in order to maintain a fixed saturation deficit at different temperatures of the growing chamber, the required difference between the temperatures of the condenser and the growing chamber is not fixed but is a mathematical function of the temperature of the growing chamber. Thus, if the growing chamber be maintained at a temperature of 14.6°C . and the air passing into this be saturated at a temperature of 10°C ., it will have a saturation deficit of 3.2 mm. on attaining the temperature of 14.6°C . If, however, the growing chamber be maintained at a temperature of 24.5°C ., the temperature of the condenser required to maintain the same saturation deficit is 22.9°C ., the difference in temperature required to maintain the saturation deficit of 3.2 mm. thus being 4.6°C . at the lower temperature and only 1.6°C . at the higher.

If, from a table of vapour pressures, one calculates the temperatures of the condenser and the growing chamber required for a particular saturation deficit at different growing chamber temperatures, and plots these in the form of a graph, it will be seen that for certain physiologically important ranges of growing chamber temperature and saturation deficit the graph approximates to a straight line; three such graphs are shown in Fig. 2.

These graphs suggest that the temperature may be treated as a linear function of the temperature of the growing chamber, and if this assumption be permissible it should be a comparatively easy matter to design a thermostatic device which would automatically maintain a constant saturation deficit in spite of temperature variations in the growing chamber. With the object of investigating this possibility the linear equations of closest fit were determined by the method of least squares for three saturation

deficits, namely 3.2 mm., 6.0 mm. and 10.0 mm.; these also are shown in Fig. 2, and it will be seen that they approach closely to the values obtained from the vapour pressure tables¹.

Having found the linear equations connecting growing chamber temperature and condenser temperature, it remained to determine the error produced in the saturation deficit by treating these equa-

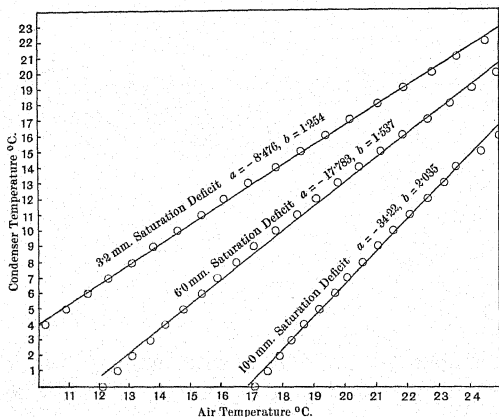


Fig. 2.

Graphs of $t_1 = a + bt_2$,

where a = constant, b = constant, t_1 = condenser temp., t_2 = air temp.

Points plotted are from Landolt's Tables.

Straight lines are linear equations of closest fit.

tions as true, and Fig. 3 shows these errors for the same three saturation deficits. It will be seen from the curves in this figure that in none of the cases considered is the error produced by treating the condenser temperature as a linear function of the temperature of the growing chamber greater than plus or minus 6.6 per cent. of the saturation deficit, except at a temperature above 24° C. with a satura-

¹ A point of some interest, although it cannot be elaborated here, is that the constants a and b in the equations appear to be approximately linear functions of the saturation deficit.

tion deficit of 10 mm. Over a shorter range of temperature than those plotted the error would obviously be less.

From these results it would appear possible to design an automatic control of saturation deficit, capable of giving constant values within about 10 per cent. total variation over a limited range of temperature; the apparatus described below has accordingly been evolved.

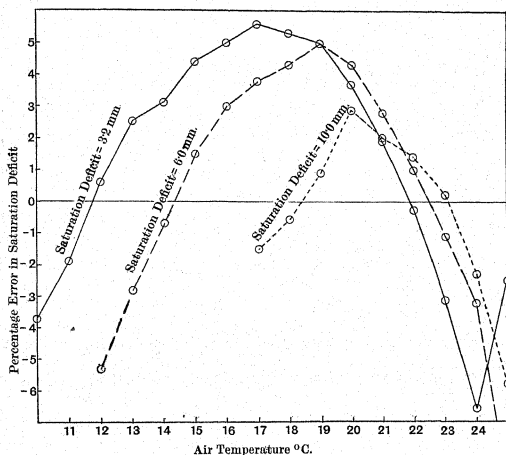


Fig. 3.

APPARATUS FOR THE CONTROL OF SATURATION DEFICIT

The apparatus is shown by Fig. 4. It consists in essentials of two mercury vessels, one *B* in the growing chamber, this vessel being so shaped as to take as nearly as practicable the mean temperature of the growing chamber; the other mercury vessel *A* is in a water bath containing the condenser, and this bath is kept well stirred by an electric stirrer (not shown). The condenser is of special design and is fitted with internal glass baffles to increase its efficiency. It is also fitted with a small glass reservoir *K*, into which condensed water drains and from which it may be drawn off by means of a tube which is normally kept closed by a rubber cap.

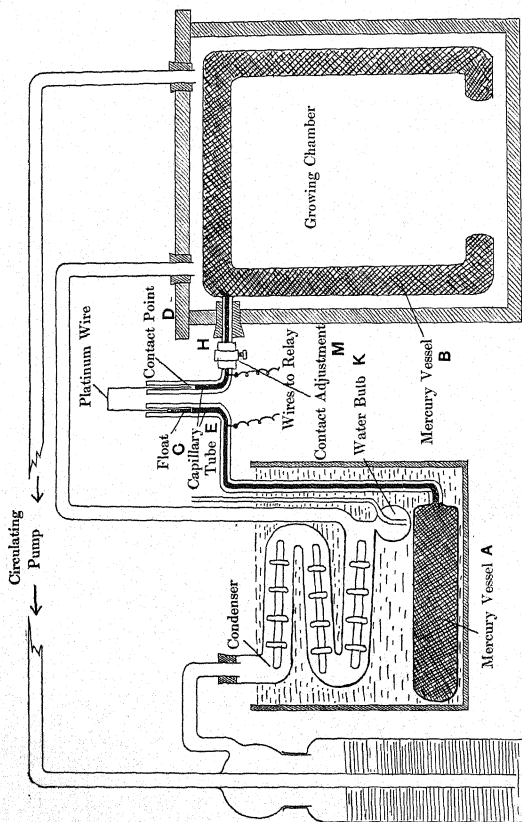


Fig. 4. Apparatus for the Control of Saturation Deficit.

Each of the mercury vessels communicates with a small vertical capillary tube, *E* and *H* in Fig. 4, these two tubes being placed side by side and securely fastened. A hollow glass float *C*, having a platinum wire passing through it into the mercury in *E*, rises and falls on the mercury meniscus in this tube, which is in communication with the mercury vessel in the condenser bath. The platinum wire is so bent as to rise and fall in the capillary tube *H* with the rise and fall of the float *C* in the capillary tube *E*. Contact with the wire is made either by a rise of mercury in *H* or by a fall of mercury in *E*. When contact is made a current passes through two wires, one sealed into each mercury system, which are connected with a battery and a sensitive relay. An electro-magnetic device (not shown) is used to control a supply of water at 0°C . to the condenser bath, the device being so constructed that when contact is made between the two mercury columns *E* and *H* the supply of cold water is shut off. A minute gas flame is kept burning under the condenser bath so that when cooling ceases the temperature rises. As soon as contact is broken by a rise of temperature in the condenser bath or a fall in the growing chamber, the cold water is again allowed to flow. The condenser bath is fitted with a constant-level overflow which is not shown. A small screw adjustment for making slight changes in the level of the mercury in tube *H* is provided and is shown by *M* in Fig. 4.

If the mercury vessels *A* and *B* were of equal capacity and the capillary tubes *E* and *H* of equal bore, it follows that the difference in temperature of condenser and growing chamber—once set by adjustment of the mercury levels in *E* and *H*—would be constant whatever the temperature of the growing chamber; if, however, the vessels *A* and *B* are of different sizes then any desired linear relations between the two temperatures can be obtained—strictly this is only true if the coefficient of expansion of mercury in glass is constant over the required range, but the error introduced by this assumption is very small.

For a saturation deficit of 3.2 it was found that the required ratio of the capacity of the vessel in the condenser bath to that of the vessel in the growing chamber was as 35.6 is to 45.0, and in the experimental apparatus the vessels were made of 35.6 c.c. and 45.0 c.c. respectively.

In practice it was found necessary to increase the rigidity of the platinum wire which rises and falls with the float *C* by surrounding it by a thin sheath of glass in the form of a very fine capillary tube.

It was also found advantageous slightly to constrict the top of the capillary tube *H* in order to prevent the platinum contact wire getting out of centre and so making contact with a portion of the mercury meniscus at a lower level, thus upsetting the adjustment; the contact wire itself was found to be more satisfactory when so bent that a minute horizontal portion of wire came in contact with the meniscus, rather than when the actual point made contact.

A preliminary trial gave the following results:—

Growing chamber	Condenser bath	Observed difference	Required difference
° C.	° C.	° C.	° C.
14.6	10.0	4.6	4.6

The apparatus was set at the above temperatures and the temperature of the growing chamber was then raised to 25.0° C.¹:—

25.0	22.35	2.65	2.4
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The apparatus was again set at:—

24.0	21.5	2.5	2.5
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The temperature of the growing chamber was then reduced¹, and two readings were obtained:—

20.5	17.15	3.35	3.2
19.6	16.0	3.6	3.4

These results, although not very accurate, indicate the value of the method; and it seems probable that the accuracy might be increased by using larger vessels. It is hoped later to publish a fuller account of the actual changes in the saturation deficit observed when using a similar type of apparatus.

¹ For the purposes of this preliminary experiment the growing chamber was filled with water and fitted with a stirrer; the temperature was changed by the addition of hot or cold water and a few minutes allowed for the attainment of equilibrium.

METHODS FOR THE STUDY OF ASSIMILATION AND RESPIRATION IN CLOSED SYSTEMS¹

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(With 5 figures in the text)

THE measurement of changes in the concentration of carbon dioxide in a closed system is a method of considerable value in the study of the respiration and assimilation of the living plant. In such a system the method used for the estimation of carbon dioxide should fulfil the following conditions:

(a) The amount of carbon dioxide withdrawn from the system for estimation should be small compared with the total amount present so that errors due to changes in concentration may be reduced to a minimum.

(b) The method should be rapid in order that changes in concentration may be followed quickly.

(c) The method should, as far as possible, be capable of giving a continuous record, thus making it practicable to determine the time taken for a change between two arbitrary limits as a means of comparison of rates of respiration or assimilation under various conditions. (Cf. Haas(1), Osterhout(5).)

A study of the available methods of carbon dioxide estimation suitable for closed systems has accordingly been made. The following methods were compared:

(1) *The Haldane Gasometric Method* of absorption in a gas pipette. This has the disadvantage that the carbon dioxide is permanently withdrawn from the system, a withdrawal which may introduce considerable errors where the circulating system is small and frequent analyses are made.

Another disadvantage of the gasometric method is that it is discontinuous and that the accurate estimation may take a considerable time if a sufficient period is allowed for the gas in the burette to attain a constant temperature and degree of humidity between each reading. On the other hand the gasometric method has the ad-

¹ Thesis approved for the Degree of Master of Science in the University of London.

vantage that it is unaffected by dust or traces of acid vapour in the air. The Haldane method, with a standardised technique, was adopted as a standard to which other methods were referred during the investigation.

(2) *Change in Electrical Conductivity.* This was determined in an aqueous solution of carbonic acid in equilibrium with the mixture of air and carbon dioxide. The method has the advantage that only small amounts of carbon dioxide are withdrawn from the system and that the successive amounts withdrawn in a series of analyses are not additive.

(3) *The Indicator Method.* This method of determining the change in hydrogen-ion concentration of the water, or of an aqueous solution of an electrolyte in equilibrium with the mixture of carbon dioxide and air, has likewise the advantage that the quantities of carbon dioxide withdrawn in successive analyses are not additive, and it has in addition the advantages of being continuous and very rapid.

These three methods were studied in detail, and determinations were made of their accuracy. The indicator method was improved and standardised.

APPARATUS USED

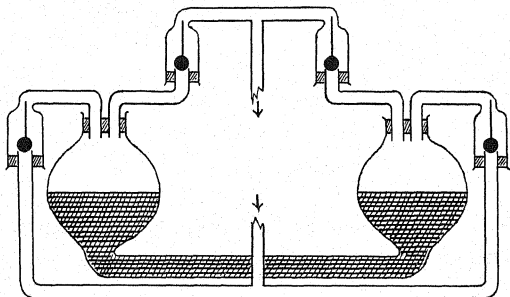
A circulating apparatus was devised, such that the mixture of carbon dioxide and air could be circulated, changed in composition, brought into equilibrium with the indicator or other solution, and analysed in the Haldane apparatus without communication of any sort with the outside air.

The pump used for circulating was specially devised to obviate the use of any gland or "stuffing-box" through which leakage of the carbon dioxide could take place. Its essential features are shown in Fig. 1. It consisted of two glass bulbs connected together by a glass tube and containing sufficient mercury to render each bulb half-full when the connecting tube was level. These two bulbs with the connecting tube were mounted on a frame which could be rocked by an excentric connected to an electric motor, the speed of which could be varied. The rise and fall of the mercury produced in the bulbs by rocking were used to take in and discharge the mixture of carbon dioxide and air through the ball valves which were made of glass and worked dry on a glass seating. The air mixture was supplied to the pump through a hollow mandrel (not shown), on which the frame rocked, the air mixture from the pump being delivered though the other end of the same mandrel. The middle portion of the mandrel was solid in order to avoid communication

between the two sides of the system. Connection with the mandrel was made by short pieces of thick-walled rubber pressure tubing, which allowed sufficient twist to accommodate the rocking motion of the pump.

In connecting the various component parts of the apparatus, which was made of glass and is shown diagrammatically in Fig. 2, the number of rubber connections was reduced to a minimum and all such connections were made with thick-walled rubber pressure tubing, the glass tubes being brought into contact within them wherever practicable.

Determinations of electrical conductivity were made in a silica cell with platinum plate electrodes, the cell being incorporated in



Not to Scale

Fig. 1. Pump used for circulating mixture of CO_2 and air.

the system when required. Measurements of resistance were made with a meter-bridge of the ordinary slider type, and various methods, which will be discussed under the heading of "Conductivity Method," were used to determine the null point.

In using the apparatus for the preliminary investigation and for standardisation, the jar (*A* in Fig. 2) containing the plant was cut out of the circuit by closing two screw clips, the vessel *B* containing the desired mixture of carbon dioxide and air was put into communication with the indicator tube or conductivity cell and with the Haldane apparatus (*C* in Fig. 2). If desired, the composition of the mixture could be changed by the introduction of a measured volume of carbon dioxide from another Haldane burette (*D* in

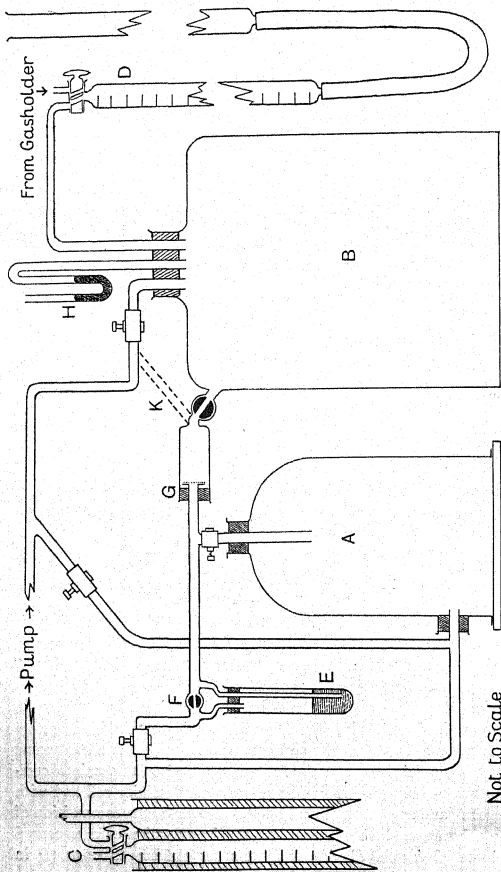


Fig. 2. General diagram of apparatus.

Fig. 2). In such cases six minutes' circulation with the pump sufficed to ensure thorough mixing, but as a precaution mixing was first effected, after introduction of the carbon dioxide, by leaving the Haldane burette used for this purpose in communication with the vessel *B* and quickly raising and lowering the mercury container, so as to fill and empty the burette about six times. It was then shut off from *B* by means of the stopcock.

When the indicator method was used, the indicator solution was placed in a "Pyrex" glass test tube, of 1 inch internal diameter (*E* in Fig. 2), through which the various mixtures of carbon dioxide and air could be bubbled; the rate of bubbling being controlled either by the speed of the pump, the degree of opening of the by-pass stopcock *F*, or both. In the later experiments on the indicator method, special devices were used to attain high accuracy in colour matching; these will be described in the account of the indicator method. A dust filter *G* was introduced into the system when using the conductivity method, *vide infra*. This dust filter was also employed in the experiments using brom-cresol purple as an indicator. A manometer (*H* in Fig. 2) was also fitted.

PREPARATION OF CARBON DIOXIDE

The carbon dioxide used was prepared by the action of dilute pure hydrochloric acid solution (concentrated one part by volume to three of water) on marble. The gas so produced was washed through a wash bottle containing a saturated solution of sodium carbonate and then through two wash bottles containing water. From this it was passed into a large wash bottle containing tap water and allowed to bubble through until the water was nearly saturated. A quantity of this water was then boiled to expel the dissolved carbon dioxide into a small mercury gas-holder. The carbon dioxide so prepared was found to consist of about 80 per cent. carbon dioxide by volume and 20 per cent. of air.

Measured volumes of this were introduced into the vessel *B* by means of the Haldane burette *D* and mixing was carried out as previously described.

When set up, the apparatus was tested by filling with various concentrations of carbon dioxide and leaving for a period of 24 hours, after which period the composition of the mixture was found to be unchanged.

THE HALDANE GASOMETRIC METHOD

This was used as the standard to which the results obtained by the indicator and the conductivity methods were referred. Certain precautions were found to be necessary in order to obtain constant results, these were:

(A) To bring the potash solution used for the absorption of carbon dioxide into equilibrium with the air before use; this was accomplished by boiling the water used before dissolving the potassium hydroxide in it, cooling after solution and shaking for some time with air before filling the absorption pipette. If these precautions were neglected a very considerable error was introduced by the potash solution either giving up dissolved air or dissolving air during the absorption.

(B) To allow time for the gas introduced into the burette to attain the temperature of the water jacket which surrounded it; and also to become saturated with water vapour. Three minutes was found to be ample.

The Haldane burette was calibrated by fusing on a glass stopcock and running out successive quantities of mercury which were weighed. Except between two particular pairs of divisions, which were avoided when using the burette, the errors in graduation were found to be well within the limit of accuracy in reading and no correction was therefore made on the percentage of carbon dioxide as given by analysis.

As a test of the accuracy of the method five successive analyses were made on a sample (20 c.c.) of atmospheric air contained within the closed circulating system. The following results were obtained:

4.5 parts carbon dioxide in 10,000.			
3.0	"	"	"
3.5	"	"	"
4.5	"	"	"
2.5	"	"	"
Mean = 3.6	"	"	"

This agrees with the degree of accuracy claimed by Haldane(2), who says that "the errors of reading should not exceed 0.001 c.c.," which gives a possible error of 0.002 c.c. in an analysis, and corresponds to plus or minus 1 part in 10,000 when using 20 c.c. of gas for analysis. The accuracy of the reading was slightly increased later by better illumination and finer marking of the absorption pipette. In this test no account was taken of the small quantities

of carbon dioxide withdrawn from the system by analysis; these amounted in all to less than 1 part in 10,000, owing to the large capacity of the vessel *B* (about 10 litres).

The gasometric method was therefore taken as being accurate to about plus or minus 1.1 parts in 10,000, and for critical work all analyses were made in duplicate and the mean taken. If these analyses differed by more than about 3 parts in 10,000 further analyses were made until agreement was obtained.

THE ELECTRICAL CONDUCTIVITY METHOD

Measurements were made of the conductivity of water in equilibrium with various mixtures of carbon dioxide and air. The water was contained in a small silica cell through which the mixture of air and carbon dioxide could be bubbled; this cell was included in the circulating system. The platinum electrodes of the cell were about 0.6 cm. diameter and about 3 cm. apart; they were not covered with platinum black, but were used bright. The cell was supported in a water bath, the temperature of which was controlled by a thermostat, and observations were made at temperatures of 20° C. and 25° C. A "buzzer" was used to generate the alternating current necessary in order to avoid polarisation, and the resistance of the liquid in the cell was determined by a Wheatstone bridge of the metre-bridge type, with a sliding contact piece, and a set of known resistances. Various methods were used to determine the null point. These were: high resistance telephones, oscillation galvanometer, thermionic amplifier with telephones, thermionic amplifier with the current rectified by a crystal and measured on an ordinary d'Arsonval galvanometer.

The best results were obtained by the last method, using an "everset" crystal of the copper-pyrites-zincite type as a rectifier. The current was passed from the bridge through an iron-cored transformer, such as is used for low-frequency amplification in wireless telephony, one end of the secondary being connected to the grid of a thermionic valve. The amplified current from the valve was passed through the primary of a second similar transformer, in order to avoid the inclusion of the galvanometer in a battery circuit, and the current induced in the secondary of the second transformer was rectified by the crystal and then passed through the galvanometer, which had a resistance of about 1300 ohms. It was found that if the ordinary galena type of crystal with a wire contact was used there was so much variation in the resistance of the

rectifier that it was impossible to determine the point of minimum deflection on balancing the bridge. With the copper-pyrites-zincite rectifier there was much less trouble from this source. The above-described method was found to be the best of those tried, but in all probability the use of a commutator with the d'Arsonval galvanometer would be superior.

A rapid fall in the resistance of the liquid in the cell was found to be caused by traces of dust in the air contained in the circulating system, and experiments were made on the filtration of the air, both for use in the conductivity method and to eliminate a possible source of error in the indicator method due to the introduction of dust.

The following results were obtained:

	Time of bubbling, Minutes	Resistance of cell, Ohms
Laboratory air unfiltered and unwashed. Temp. 25° C.	0	224,000
	120	64,000
Laboratory air bubbled through distilled water and then filtered through closely packed dry cotton-wool. Temp. 20° C.	60	367,000
	120	382,000
	180	374,000
	240	277,000
Laboratory air, washed, and filtered through wet glass wool. Temp. 25° C.	0	395,000
	220	394,000
	300	175,000
	Hours	
Laboratory air, filtered through dry filter paper of close texture, as used by the Meteorological Office for dust estimations. Air unwashed. Temp. 25° C.	0·0	413,000
	1·5	410,500
Water left in cell for two days, not bubbling		
	1·5	441,500
	7·0	412,000

Filtration through thick filter paper thus appears to be a satisfactory method for the removal of dust, and was used in the later experiments on indicators.

The error of the measurement of resistance of the cell was found by a series of consecutive determinations to be about 30,000 ohms to 40,000 ohms. This was somewhat reduced in most cases by taking the mean of several readings.

The effect of different mixtures of carbon dioxide and air was studied, and the following values for the resistance of the cell were obtained:

Carbon dioxide in equilibrium with water at 20° C. Air unwashed and unfiltered. Single determinations of resistance

Time of bubbling Minutes	Analysis by gasometric method	
	Parts of carbon dioxide in 10,000 of air	Resistance of cell Ohms
0	5.0	442,000
12	10.0	407,000
54	16.5	353,000
74	31.0	287,000

This work was carried out before the need for filtration of the air was realised, but in the light of the work on the effect of dust, already given, it seems certain that the greater part of this fall in resistance is due to dust. Even if it were all due to changes in the amount of dissolved carbon dioxide the method would not be so accurate as the indicator method to be described later. Further work on the electrical conductivity method was therefore abandoned.

THE INDICATOR METHOD

This depends on the colour change, due to change in hydrogen-ion concentration, of an aqueous solution of an indicator in equilibrium with air containing various concentrations of carbon dioxide.

In all these experiments distilled water of good quality, prepared by condensation in a tin condenser, collection in a stoneware vessel, and storage in "Pyrex" flasks, was used.

A preliminary determination of the *pH* range required for water in equilibrium with various concentrations of carbon dioxide in air, from 0 to 40 parts in 10,000, suggested that methyl red, having a *pH* range from 6.0 to 4.4, was the most suitable indicator, but the method of Higgins and Mariott(4), using an aqueous solution of phenol red buffered with sodium carbonate, was first investigated. The indicators used were obtained from the British Drug Houses, Ltd.

The method of Higgins and Mariott when tested did not appear to be as sensitive as claimed by the authors, thus a mixture of carbon dioxide and air containing 25.5 parts of carbon dioxide in 10,000 of air was found on analysis by the method of Higgins and Mariott to correspond with their buffer solutions indicating 10 to 15 parts. The method was therefore rejected in favour of the more sensitive methods using methyl red or brom-cresol purple.

The methyl red indicator was found to be the most sensitive, but the stock solution of methyl red used for dilution (Clark(3)), and the diluted solution used in the indicator tube, were both too unstable for use with an aqueous solution of carbon dioxide, although

the indicator was more stable when in the sodium-phosphate-acid-phosphate buffer solutions used to give the colour standards. When used in an aqueous solution of carbonic acid a perceptable change in the colour relations was sometimes found half an hour after dilution.

Attempts to overcome this difficulty were made by using the acid methyl red in alcoholic solution as a stock, and also by using the sodium salt of methyl red in alcoholic solutions of various concentrations, but without success.

Various derivatives of methyl red were prepared in the attempt to obtain an indicator of the methyl red type, which would combine the high sensitiveness of methyl red with stability, but without any marked degree of success; the methyl red type of indicator was therefore rejected.

Other indicators were tried both in aqueous and in dilute alcoholic solutions; in the case of the dilute alcoholic solutions alcohol was used as a solvent for the indicator, a few drops of the alcoholic solution being added to 10 c.c. of water to form the indicator solution used. The dyes were purchased in the dry state from the British Drug Houses, Ltd. The results are summarised below:

Indicator	Remarks
<i>p</i> -Nitrophenol, aqueous	Change from colourless to yellow, too slight and difficult to match
<i>p</i> -Nitrophenol, alcoholic	" " " "
Resaurine, aqueous	Colour change too slight
Resaurine, alcoholic	" "
Phenacetolin, aqueous	" "
Brom-thymol blue, sodium salt, aqueous	Requires too much buffering to cover desired range, when so buffered becomes insensitive
Brom-thymol blue, acid dye, alcoholic	" " " "
Brom-cresol purple, sodium salt, aqueous	Good when suitably buffered and is very stable
Phenol red, aqueous	Fair when suitably buffered

There does not appear to be a very great difference between the sensitiveness of phenol red and brom-cresol purple, but brom-cresol purple was chosen as having a natural range (*pH* 5.4 to 7.0) somewhat nearer to that of methyl red (*pH* 4.4 to 6.0) than phenol red (*pH* 6.6 to 8.2) and so requiring less buffering.

Brom-cresol purple being thus, on general grounds, the most suitable of the indicators tried, it became necessary so to modify the technique as to obtain the best results, and the first consideration

was the means of obtaining the colour standards and accurate matching; the latter being of great importance in this connection as the amount of colour change for a given change in carbon dioxide concentration is considerably less with brom-cresol purple than with methyl red.

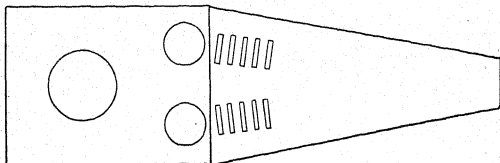
Attempts were made to obtain colour standards for various concentrations of carbon dioxide by means of phosphate-acid-phosphate buffers, but it was found that the colour of the brom-cresol purple when buffered with sodium carbonate and in equilibrium with carbon dioxide was very different qualitatively from the colour in such a buffer solution, thus making these useless as colour standards. As a carbonate-bicarbonate buffer was considered to be too unstable for a colour standard, another means of obtaining a standard was devised.

The colour standards finally adopted were determined in terms of red, blue and yellow, by means of a series of coloured glass slips of these colours, the slips consisting of colourless glass "flashed" with various thicknesses of coloured glass. These glasses were so adjusted that the sum of the colour values in any one series of slips exactly matched one slip having a numerical value equal to the sum; in addition the slips were so prepared that equal numerical values of the three colours, red, blue and yellow, when superimposed gave a neutral grey. The colour being due to metallic substances in the glass, no change was to be anticipated even in the course of a number of years if not unduly exposed to daylight. Such a series of glass slips was obtained from Baird and Tatlock, London, and by this means very accurate matchings of the colours, in this case from purple to yellow, were obtained.

Colour matchings were made in the light from a "Mazda" "day-light" lamp having a current consumption of 60 watts, the light being reflected from a sheet of good quality white opal glass. It was found essential to use the same lamp throughout the experiments, as different bulbs of the same make, and current consumption gave different colour values. The indicator solution was contained in "Pyrex" glass tubes of 20 mm. internal diameter, and behind the colour slips used for matching the indicator was placed a similar tube containing water. These were maintained at a constant temperature in a water bath, the temperature of which could be kept constant within 0.1°C ., this variation having been found to make no difference in the visual values of the colours. A slip of colourless glass was placed in front of the tube containing the indicator, in order to make conditions of matching as nearly as possible similar.

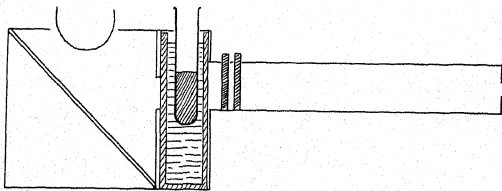
The water bath and the tubes were enclosed in a light-tight box, containing the lamp, reflector, and a series of slots for holding the coloured glasses. Fig. 3 shows the top view of the box with the slots for the coloured glasses and the holes for the "Pyrex" tubes; Fig. 4 shows a section of the box in side view with water bath and reflector chamber.

The stock solution of brom-cresol purple was prepared by making a 0.4 per cent. solution of the sodium salt, which was diluted for use. As it was considered that different samples of the dye would



Not to Scale

Fig. 3. Top view of box showing slots for coloured glasses and holes for tubes.



Not to Scale

Fig. 4. Section of box as in Fig. 3.

not be so exactly similar as to obviate the necessity for standardisation of each stock solution, a considerable quantity of the stock solution was prepared, filtered to remove suspended matter—which also changed the amount of dye in solution, but as the standardisation was carried out after filtration this was of no importance—and then standardised. The first stock solution used was prepared thus:

Brom-cresol purple, solid	0.4 gm.
Sodium hydroxide <i>M</i> /10	7.4 c.c.

The dye and the sodium hydroxide were ground together in an agate mortar and the solution was then made up to 100 c.c. with

distilled water. This gives the 0.4 per cent. solution as recommended by Clark(3), which was then filtered through a Whatman's No. 1 filter paper of 15 cm. diameter.

The solution so prepared did not undergo any change in its colour values in a period of three and a half months.

A stock solution of $M/100$ sodium carbonate was also prepared.

The indicator used in the tube was made from the two stock solutions as given below, numerous trials having shown that the concentration of indicator and degree of buffering so obtained gave the highest degree of accuracy over the desired range, i.e. 2.5 parts to 35.0 parts of carbon dioxide in 10,000 of air:

Brom-cresol purple, stock solution diluted to five times its volume	0.2 c.c.
Sodium carbonate, stock solution diluted to ten times its volume	2.0 c.c.
Distilled water	10.0 c.c.

Making a total volume of 12.2 c.c. which was used in the indicator tube.

Bubbling for one and a half minutes at a rate of about 200 c.c. per minute was usually sufficient to establish equilibrium between the indicator and the mixture of carbon dioxide and air, unless the change in the concentration of carbon dioxide was more than about 20 parts in 10,000, when about three minutes was necessary. For small changes in concentration bubbling was therefore allowed to continue for three minutes before matching, while for larger changes it was allowed to continue for six minutes. As a check, matchings were made after bubbling for six minutes and twelve minutes respectively but no further change in colour was found. All matchings were carried out at a temperature of 25° C.

When matched in the light from the "daylight" lamp, the following relations were obtained between the standard colour slips and the concentration of carbon dioxide:

Parts carbon dioxide in 10,000 (Analysis by gasometric method)	Colour slips		
	Red	Yellow	Blue
2.5	8.0	0.0	4.5
3.5	8.0	0.0	4.0
5.0	7.0	0.0	3.5
9.5	6.0	0.0	3.0
25.0	5.0	0.5	2.0
29.0	4.5	0.0	1.5
32.5	4.5	less than 0.5	1.5
33.5	4.0	0.5	1.5

It was found that intermediate values could be determined with considerable accuracy on this basis.

In order to investigate the stability of the dilute solution of the indicator as used in the tube, determinations were made after the indicator had been standing in the tube for one month, and the results indicated a decrease in sensitiveness, this decrease corresponding to from 3 to 10 parts of carbon dioxide in 10,000 according to the concentration at which it was determined. The table given below shows the experimental values obtained.

Parts carbon dioxide in 10,000 (Gasometric analysis)	Colour slips					
	(fresh indicator)			(indicator one month in tube)		
	Red	Yellow	Blue	Red	Yellow	Blue
5.0	7.0	0.0	3.0	—	—	—
6.5	—	—	—	8.0	0.0	4.0
25.0	5.0	0.5	2.0	5.5	0.5	2.5
33.5	4.0	0.5	1.5	—	—	—
35.0	—	—	—	5.0	0.5	2.0

APPLICATION OF THE METHOD

An experiment was made with a cut shoot of *Vicia faba* standing in water, the object being to ascertain whether any acids or other substances were produced by the plant, which would interfere with the accuracy of the method, vessel *B* being cut out of circuit and a by-pass fitted as shown by dotted lines at *K* (see Fig. 2).

In all experiments with brom-cresol purple a paper dust filter, as previously described, was incorporated in the circulating system.

Shoot of Vicia faba in closed system. Indicator temperature 25° C.

Vessel B cut out of circuit

(Middle of plant was 12 inches from 200 watt gas-filled lamp)

Time of shoot in closed system, Minutes	Carbon dioxide in 10,000 (Gasometric)	Colour slips			Carbon dioxide (by colour)
		Red	Yellow	Blue	
0	—	4.5+	0.0	1.5	28.0
12	25.0	5.0	0.5	2.0	25.0
198	20.0	5.5	0.0	2.0	20.0

System left closed for two days in very faint daylight; too much carbon dioxide for matching; reduced by admixture with atmospheric air:

2 days	33.5	4.5	0.5	1.5	32.5
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This preliminary trial suggested that the required method had been achieved. More careful experiments were therefore carried

out with another stock solution of brom-cresol purple, the numerical colour values for red, yellow and blue slips being separately plotted against carbon dioxide concentration and curves drawn showing the relation of colour to concentration (Fig. 5). The points found did not always lie on the curves drawn through the mean values of the separate colours for different concentrations, and when the colours required for matching did not lie exactly on the curves the part of each curve corresponding to the value of that colour required for matching was found by inspection and the concentration corresponding to this part of the curve was read off. This was done for each of the

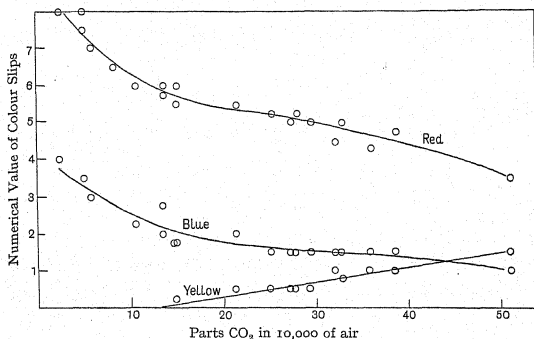


Fig. 5. Curves showing means of all colour values obtained with particular stock solutions of indicator and of sodium carbonate.

three curves, and the mean of these three values for the concentration of carbon dioxide present was taken as giving the true amount.

In all the estimations given below, at least two analyses of the mixtures were made by the Haldane method and if these differed by more than three parts of carbon dioxide in 10,000 of air further analyses were made.

In order to remove any mercury vapour carried over by the pump, thus producing toxic effects in the plant, the air was passed through a glass tube packed with gold leaf: this tube which also contains the dust filter is shown at G in Fig. 2.

The curves shown in Fig. 5 represent the mean of all colour values obtained with one particular stock solution of indicator and

of sodium carbonate, whether with the plant in the system or not. The differences in concentration as determined by the indicator method and by the Haldane method are shown in the tables given below.

For these determinations a stock solution similar to that used for the preliminary experiments was employed, but it was made from another sample of brom-cresol purple, also obtained from the British Drug Houses, Ltd. The actual quantities taken were:

Brom-cresol purple, solid	0.5 gm.
Sodium hydroxide <i>M</i> /10	9.25 c.c.

These were ground together in an agate mortar, made up to 125 c.c. with distilled water, thus giving a 0.4 per cent. solution, as used before, and filtered three times through one Whatman's No. 1 filter paper of 15 cm. diameter. A fresh stock solution of *M*/100 sodium carbonate was also prepared, thus:

Sodium carbonate, pure, anhydrous	1.06 gm.
Distilled water to	1 litre

From these the indicator was prepared as before for use in the tube:

Stock brom-cresol purple, diluted to five times	
its volume	0.2 c.c.
Stock sodium carbonate, diluted to ten times	
its volume	2.0 c.c.
Distilled water	10.0 c.c.

Except where otherwise stated in the tables, the indicator in the tube was replaced each day, no change in the diluted indicator having been observed in a period of eight hours. All matchings were made with the indicator at 25° C.

Carbon dioxide introduced from gasholder. No plant in system

Carbon dioxide in 10,000.	Colour slips			Carbon dioxide estimated from mean of colour curves	Error of colorimetric method; parts in 10,000
Mean of two or more gasometric analyses	Red	Yellow	Blue		
2.5	8.0	0.0	4.0	1.75	0.75
5.0	7.5	0.0	3.5	3.87	1.51
5.75	7.0	0.0	3.0	6.5	0.75
13.25	6.0	0.0	2.75	10.25	3.0
14.5	5.5	0.0	1.75	18.25	4.25
21.25	5.5	0.5	2.0	19.17	2.08
29.25	5.0	0.5	1.5	28.33	0.92
32.75	5.0	0.75	1.5	30.5	2.25
38.5	4.75	1.0	1.5	34.0	4.5

Cut shoot of Eupatorium with six leaves set up in system at

12 noon, Nov. 3rd. Plant in darkness

Vessel B cut out of circuit

Time	Carbon dioxide, gasometric, mean of two or more analyses	Colour slips			Carbon dioxide from mean of colour curves	Error of colorimetric method in parts in 10,000
		Red	Yellow	Blue		
<i>Nov. 3rd</i>						
2.30 p.m.	10.25	6.0	0.0	2.25	12.37	2.12
4.20 p.m.	13.25	5.75	0.0	2.0	14.62	1.37
<i>Nov. 4th</i>						
11.30 a.m.	25.0	5.25	0.5	1.5	26.33	1.33
4.0 p.m.	27.7	5.25	0.5	1.5	26.33	1.37
<i>Nov. 5th</i>						
10.30 a.m.	32.0	4.5	1.0	1.5	34.91	2.91
4.0 p.m.	35.75	4.35	1.0	1.5	35.53	0.22
<i>Nov. 7th</i>						
11.30 a.m.	51.0	3.5	1.5	1.0	—	—
12 noon	Plant exposed to light from 200 watt gas-filled lamp at a distance of one foot from middle of plant. Left illuminated until					
<i>Nov. 8th</i>						
11.15 a.m.	8.25	6.5	0.0	2.5	9.75	1.5

Cut shoot of Eupatorium with eight leaves set up in system at

2.5 p.m. on Oct. 6th. Plant in darkness

Vessel B cut out of circuit

Time	Carbon dioxide, gasometric, mean of two or more analyses	Colour slips			Carbon dioxide from mean of colour curves	Error of colorimetric method in parts in 10,000
		Red	Yellow	Blue		
<i>Oct. 6th</i>						
3.30 p.m.	5.0	8.0	0.0	3.5	2.87	2.13
4.0 p.m.	Plant illuminated by 200 watt gas-filled lamp at 20 inches from middle of plant until					
<i>Oct. 7th</i>						
		(no fresh indicator)				
9.30 a.m.	1.5	8.5	0.0	4.0	1.0	0.5
9.35 a.m.	Plant darkened:					
<i>Oct. 8th</i>						
		(no fresh indicator)				
9.45 a.m.	16.25	6.0	0.0	1.75	15.5	0.75
<i>Oct. 9th</i>						
		(no fresh indicator)				
9.40 a.m.	29.0	5.25	0.5	1.5	26.33	2.67
12 noon	A little carbon dioxide allowed to escape in changing the indicator solution.					
		(fresh indicator)				
12.5 p.m.	27.0	5.0	0.5	1.5	28.33	1.33
12.15 p.m.	Plant exposed to light from 200 watt gas-filled lamp at 12 inches from middle of plant until					
3.15 p.m.	14.75	6.0	0.25	1.75	16.66	1.91

These results demonstrate that the indicator method is suitable for quick estimations of carbon dioxide in air when the concentrations are between 5 parts and 35 parts in 10,000. The mean error of the 20 results given in the above tables (those with the stale indicator being excluded) is 1.85 parts in 10,000. In only two of the estimations was the error, as determined by comparison with the gasometric method, greater than 3 parts in 10,000: these two errors, 4.5 parts and 4.25 parts, may be considered as exceptional.

SUMMARY

An apparatus is devised for the preparation of carbon dioxide mixtures and their circulation in a closed system containing a living plant. The system includes a simple mercury pump having glass ball valves, working without grease and avoiding the risk of leakage due to packing of a moving part.

Using this system, three methods of carbon dioxide estimation have been compared, the Haldane gasometric method, the electrical conductivity method, and the indicator method.

The indicator method when improved and standardised is found to be suitable for rapid determination of carbon dioxide in the study of respiration and assimilation. The most satisfactory indicator is found to be brom-cresol purple in the form of its sodium salt and buffered with sodium carbonate, in aqueous solution. The indicator colour is matched against coloured glass slips under constant lighting conditions.

No evidence is obtained that cut shoots of *Vicia faba* or of *Eupatorium* sp. give off volatile products which introduce any error.

This work was undertaken at the suggestion of Professor V. H. Blackman, of whose interest and kindly supervision I wish to express my sincere appreciation.

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THE NEW PHYTOLOGIST

VOL. XXV, No. 3

31 JULY, 1926

LIGHT AND GROWTH

II. ON THE ANATOMY OF ETIOLATED PLANTS

By J. H. PRIESTLEY

(With Plates IV and V and 7 figures in the text)

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INTRODUCTION

SINCE the preliminary account of etiolation (12) was published from the Botanical Department of the University of Leeds, further observations have been added in later papers, on the cuticle (9), on the effect of ethylene (13), on the small production of wound cork in response to superficial wounds (14) in etiolated plants, and on the structural response to etiolation given by many Monocotyledons (22). Indeed, work upon etiolation has been practically continuous since the earlier experiments were recorded, thanks to a succession of research students, and particularly to the co-operation of two research assistants, Miss R. M. Tupper-Carey and Miss Ursula Tetley.

The observations which follow have been made as the result of many and various methods of investigation, but they seem to have a common focus. These methods have all been finally employed in the examination of plants grown under the controlled conditions described in the first paper (15) of the present series on "Light and Growth." Such methods are not readily applied on a very large scale, so the number of species of plants examined is small, and usually the data given in the following account were obtained from *Vicia Faba* L. and *Pisum sativum* L. These plants show a very

characteristic response to growth in darkness, which is typical of the majority of Dicotyledonous plants.

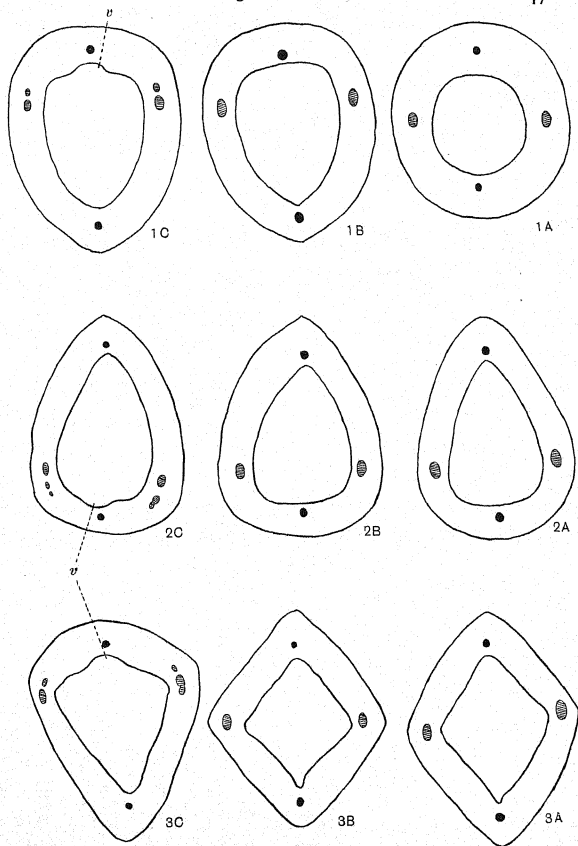
The interpretation of the structural changes, produced in the plant by growth in darkness, advanced in the discussion, seems to follow naturally from the facts recorded in the experimental section, and the different growth responses of other plants when etiolated may find a solution along similar lines.

THE GENERAL ANATOMY OF THE NORMAL AND ETIOLATED PLANT
OF *VICIA FABA* L.

Data drawn from the broad bean are to be utilised so frequently that the general anatomy of the normal plant requires brief discussion first. It has been described in detail already by Goldschmidt(7) and Compton(3). The normal bean stem always bears scale leaves at the first two nodes, above this level the stem is square in cross section, and the fully developed leaves arise alternately on opposite sides of the stem.

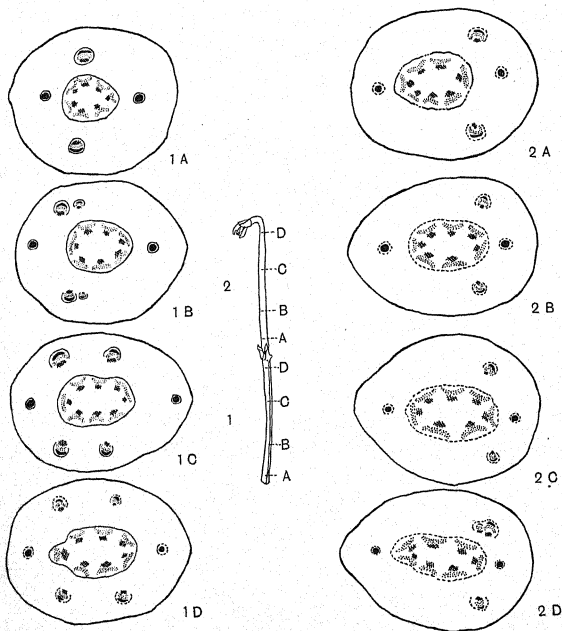
A series of transverse sections through the stem is shown in Text-fig. 1, which elucidates the supply of the vascular system to the leaf.

At the base of an internode there are, in the cortex, two vascular bundles which run up towards the leaf above, and between these (1 *A* in Text-fig. 1) there are, in addition, two cortical strands of sclerenchyma which have also broken away from the stele at the node below, the cotyledonary node in the case of the first internode. Just below the node above (1 *C*, 2 *C* of Text-fig. 1), these vascular bundles divide into two or more bundles, the main group of bundles thus formed turning outwards into the leaf, where they form the two wings of the arc of bundles which enters the petiole, the main mass of bundles coming straight out into the petiole from the stele at the node (*v* in Text-fig. 1) and being subtended by one of the strands of sclerenchyma from the cortex, which runs along the lower side of the main vein of the leaf. Immediately above the node there are left, therefore, one bundle of sclerenchyma on the side of the stem opposite the leaf insertion, and two cortical vascular bundles, the branches given off by the pair which have gone to form the petiolar wings. Another bundle of sclerenchyma splits off from the stele immediately in the axil of the leaf (2 *A* or 3 *A* of Text-fig. 1), and thus there are again present two vascular bundles, and, in a plane at right angles to these, two strands of sclerenchyma. Four cortical strands are found at the angles of a square stem practically from the base of the second internode, and the vascular system is



Text-fig. 1. Series of sections through three internodes of a normal broad bean (*Vicia Faba*) stem. The internodes are numbered from base to apex. A, B, C=position of section in internode. A is just above the node, C is just below next node, B is mid-way between A and C. The inner line shows the outline of the stele. Cortical vascular bundles shaded; sclerenchyma black; v, part of main stele passing out to supply petiole.

arranged in a manner which persists in all succeeding internodes. The base of the lowest internode, on the other hand, is not square in outline and the cortical strands are not so near the surface.



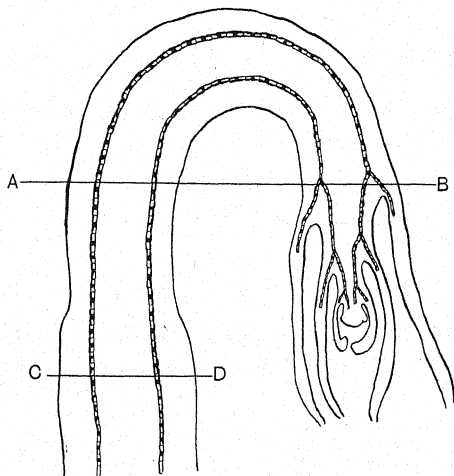
Text-fig. 2. Series of sections through two internodes of an etiolated bean stem. The internodes are numbered and lettered as in Fig. 1, position of the section indicated in the diagram of two internodes of plant. Sclerenchyma black; phloem dotted; xylem cross-hatched; primary endodermis represented by a continuous line; starch sheath by an interrupted line.

A comparable series of figures for the first two internodes of the etiolated plant is shown in Text-fig. 2.

In comparison with the normal plant it will be observed that

the internodes, which are of course much longer, never become fully square in section, and only approach this outline (1 *D* and 2 *D* of Text-fig. 2) just below the base of the internode. The cortical vascular bundles and sclerenchyma strands lie deeper in the cortex and are not associated with angular projections on the surface.

A constant feature of the basal internode of the etiolated plant is the fact that the cortical bundles seem to divide relatively much earlier

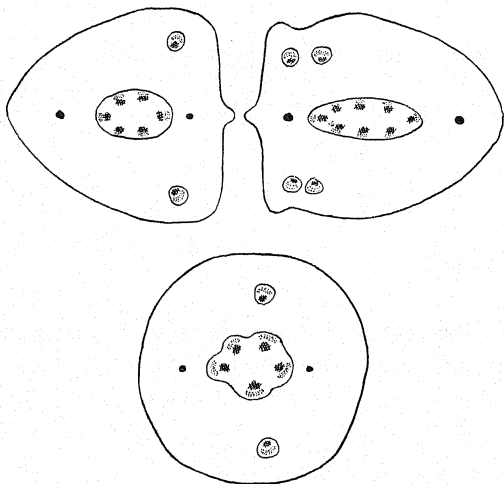


Text-fig. 3. L.S. of plumular hook of broad bean, showing course of vascular bundles forming main stele.

than in the non-etiolated plant, so that two bundles can be traced on either flank of the cortex through the greater part of the internode.

When the etiolated seedling has developed the first two internodes, the third will be found curled in the plumular hook. In this internode the vascular strands can be traced right round the hook (Text-fig. 3), the xylem being differentiated to some extent in the bundles, particularly on the outer side of the hook, on which side the trace will pass out from the next node into the leaf; these two cortical vascular bundles lie on the flanks towards the inner side of

the hook (Text-fig. 4). The procambial strands of this leaf trace and of the one next above are already present. The cortical sclerenchyma group on the outer side of the hook is also more strongly developed than that on the inner side. Even at an earlier stage of the development of the seedling, when the second scale leaf had only recently parted from the bud and was moving slowly down along the *inner* side of the plumular hook, the vascular strand on the outer side of of



Text-fig. 4. Transverse sections of plumular hook, upper one taken at *AB*, lower at *CD* in text-fig. 3. Phloem areas dotted; xylem cross-hatched; sclerenchyma black.

the stele was already more fully developed than that on the inner. This is probably associated with the more fully developed leaf rudiment that is present on the outer side of the hook, as compared with the slightly developed scale leaf on the inner side. The relative vascular development on the opposite sides of the hook has undoubtedly considerable significance in relation to the existence of the hook, and will be considered in this connection in the discussion.

From Text-figs. 3 and 4 it will be seen that the vascular strands are laid down upon the typical plan, and the vascular bundles to just below the third node are already divided, so that strands for the cortex of the fourth internode are present. These further leaf initials never appear however to make any further step in development in a completely etiolated plant, but persist at the stage of development thus indicated. On the other hand with even a short light exposure, such as two minutes each twenty-four hours to about one thousand metre candles, complete disappearance of the plumular hook occurs, these meristematic leaves undergo further differentiation, and the third and further internodes may develop with the usual type of vascular anatomy; although, as shown in the next section, the relative dimensions of stele and cortex may not be quite typical of the normal plant.

THE RELATIVE SIZE OF STELE AND STEM

Another very marked difference between normal and etiolated plants is the relative dimensions of the central vascular cylinder, as delimited by endodermis or starch sheath. This vascular stele is always smaller in diameter, and has a more definite *ring* of vascular elements in the etiolated plant. In order to give precision to this statement, data are presented in Tables I and II for *Vicia* and *Pisum*, which were obtained by Miss Ursula Tetley in the following manner.

Sections of the stem at definite positions were projected on to paper by a microscope projection apparatus, and the outline of stem and stele then traced and transferred to squared paper. The resulting data, given in square millimetres as the paper was ruled, are of course in arbitrary units. As the sections were magnified approximately thirty times, those arbitrary units can be roughly converted into square millimetres by division by 900.

In the first column of this table the number in brackets after the number of the internode indicates the position. Thus (1) was just above the node, (4) just below the next node, (2) one-third of the way up the internode, (2.5) half, and (3) two-thirds of the way up the internode. In Table II, the data for positions (1) and (4) are not given, as the stele dilates so markedly in the neighbourhood of the node that consistent results could not be obtained for comparison. Duplicate readings for different sections at the same level are frequently given so that an estimate may be formed of the value of these data for purposes of comparison.

TABLE I
Dimensions of Stele and Stem. *Vicia Faba* L.

Internode	Area of transverse section of		Area of transverse section of stem expressed as a multiple of that of the stele (taken as unity)	
	Stele	Stem		
1. <i>Normal Bean Stem. 18 days old</i>				
I (1)	420	3020	7.2	7.15
I (1)	430	3049	7.1	
I (2.5)	463	2985	6.4	6.35
I (2.5)	463	2940	6.3	
I (4)	552	2840	5.1	5.0
I (4)	606	2995	4.9	
2 (1)	495	2345	4.7	4.8
2 (1)	458	2335	5.0	
2 (1)	498	2388	4.7	4.55
2 (2.5)	505	2324	4.6	
2 (2.5)	498	2244	4.5	4.55
2 (4)	642	2446	3.6	
2 (4)	660	2463	3.7	3.65
3 (1)	575	1749	3.0	
2. <i>Plants given 1 hour daylight per day</i>				
I (1)	335	3169	9.3	9.36
I (1)	338	3184	9.4	
I (1)	309	2848	9.4	7.9
I (2.5)	327	2564	7.8	
I (2.5)	301	2547	7.9	7.9
I (2.5)	325	2603	8.0	
I (4)	541	3425	6.3	6.2
I (4)	559	3413	6.1	
2 (1)	524	2598	4.9	4.85
2 (1)	545	2623	4.8	
2 (2.5)	511	2294	4.5	4.45
2 (2.5)	525	2292	4.4	
2 (4)	630	2305	3.6	3.65
2 (4)	615	2292	3.7	
3. <i>Etiolated Plant</i>				
I (1)	194	2000	10.3	10.25
I (1)	187	1935	10.2	
I (2)	179	2285	12.2	9.0
I (3)	227	2087	9.0	
I (4)	334	2168	6.0	8.9
2 (1)	226	2014	8.9	
2 (2)	221	1629	7.4	6.95
2 (3)	231	1644	7.1	
2 (3)	225	1559	6.8	5.5
2 (4)	199	1113	5.5	
2 (4)	207	1130	5.5	5.5

TABLE II
Areas of Stele and Stem. *Pisum sativum* L.

Internode	Area of transverse section of		Area of transverse section of stem expressed as a multiple of that of the stele (taken as unity)
	Stele	Stem	
1. <i>Normal Plant</i>			
1 (2)	109	1339	14.1
	101	1398	13.9
1 (3)	157	1681	10.6
	151	1647	10.8
2 (2)	133	1592	12.0
	129	1543	11.9
2 (3)	160	1415	8.8
	158	1423	9.0
	162	1415	8.7
2. <i>Plants given 1 hour daylight each day</i>			
1 (2)	93	1666	17.0
	100	1711	17.1
1 (3)	105	1352	12.9
	107	1357	12.7
	102	1322	12.9
2 (2)	100	1041	10.4
	100	1043	10.4
2 (3)	122	1211	9.9
	125	1216	9.8
	122	1235	10.0
3. <i>Etiolated Plant</i>			
1 (2)	85	1567	18.0
	92	1637	17.8
	91	1625	17.8
1 (3)	100	1547	15.4
	100	1538	15.3
	107	1576	15.4
2 (2)	85	1241	14.5
	82	1200	14.6
2 (3)	114	1502	13.1
	110	1475	13.4
	108	1464	13.5

In any such comparison the absolute sizes of the plants can hardly be considered as there is always great difference amongst the individual plants, and on the whole no significant differences are indicated in columns 2 and 3 which supply data as to the actual area of stele and stem. In column 4, however, where these figures are reduced to a ratio, the area of the stele being expressed as unity, a very distinct tendency can be noted. In both plants, and with

great uniformity, the stele proves to be relatively larger in the normal plant, that is the figure for the area of stem, compared with the stele as unity, is smaller in the normal plant. This difference is consistent, and still more striking is the fact that in every case, the effect of one hour illumination per day is to give an intermediate set of figures. In each case, however, the ratio of stem to stele area in the plants exposed to one hour's light per day, though smaller than in those grown in normal light, approximates more closely to the latter than to that of the etiolated plants.

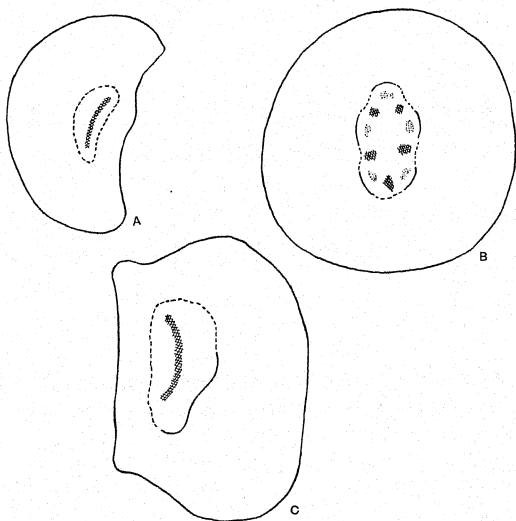
ENDODERMIS AND STARCH SHEATH

As pointed out in a previous paper (12), one of the most striking differences between etiolated and normal stems, is the delimitation of the stele in the shoot of the etiolated plant by a typical endodermis with Casparian strip. The endodermis may be present at the very base of the stem in the normal plant, but is absent throughout the greater part of the epicotyl. This difference is constant in such species of *Vicia* and *Pisum* as have been examined and in very many other genera of Dicotyledons, but in *Phaseolus* the etiolated epicotyl is without an endodermis, whilst, for instance, in many of the Labiatae the normal light-grown stem contains a typical primary endodermis.

Further study has made the following point clear. In the etiolated shoot of *Vicia* or *Pisum*, if a very young shoot is examined, no primary endodermis can be found in the plumule (Text-fig. 6), but well marked starch sheaths surround both the main stele and the cortical bundles.

Reference to Text-fig. 1 will show that later the starch sheath in all these places is replaced by a normal primary endodermis with Casparian strip, and from this endodermis all starch grains have disappeared. As the endodermis is traced upwards it will be found to disappear gradually (Text-fig. 2), being lost first, sometimes from the bundle of sclerenchyma, sometimes from the cortical bundles. In the main stele, apart from interruption at the node, it lasts considerably farther up the shoot. In all cases, when it finally disappears, it is replaced by a starch sheath. In the region of this transition cells will often be seen containing the Casparian strip and a few starch grains, and it is clear from the study of development that throughout the shoot, during germination in darkness, a transition from starch sheath to primary endodermis progresses in an acropetal direction.

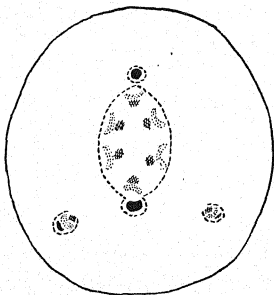
In a young seedling before any trace of endodermis with Casparian strip can be detected replacing the starch sheath of the stem, a Casparian strip may already be present in the radicle (Text-fig. 5). Appearing first immediately behind the growing point, and, in some cases, in patches opposite the differentiating phloem groups only,



Text-fig. 5. *A*, T.S. of cotyledon stalk of broad bean with plumule undeveloped. *B*, T.S. of radicle (7 cm. from tip) of the same. *C*, T.S. of cotyledon stalk of broad bean grown in continuous light showing a discontinuous primary endodermis. The limiting layer between cortex and stele is shown by an *interrupted line*, except where there is a primary endodermis, which is shown by a *continuous line*; xylem *cross-hatched*; phloem *dotted*.

it soon forms a continuous ring and can be traced upward into the hypocotyl, appearing part of the way around the cotyledonary traces and as a continuous ring around the stele at the base of the young epicotyl. The endodermis around the cotyledonary trace is much more developed where the trace joins the plumule than towards the cotyledon (Text-fig. 5 *A* and 5 *C*).

In fact in its appearance here there is nothing to contradict the conclusion arrived at earlier (18, p. 124) that the deposition of a Casparian strip occurs when the differentiation of the stele synchronises with the differentiation of the tissue in the endodermal region. Where the cells in the close neighbourhood of the stele are laid down much earlier and do not continue to divide and grow as the stele differentiates, as in the main part of the cotyledon, then no true endodermis with Casparian strip develops. When the radicle is sufficiently developed to contribute towards the maintenance of an exudation pressure probably a considerable drive of sap begins in the vascular system, driving past the cotyledonary traces into the rudimentary



Text-fig. 6. T.S. of plumule of etiolated pea, 4 days old. The starch sheaths are shown by interrupted lines. Phloem dotted; xylem cross-hatched; sclerenchyma black.

system of the still dormant plumule. This is followed by considerable growth activities of the shoot apex, and now the acropetal development of an endodermis begins from the very base of the etiolated epicotyl.

But one important difference is a constant feature of endodermal development in plumule and shoot as compared with that of radicle and root. Whilst in the shoot the Casparian strip is deposited on the radial and transverse walls of the cells of a definite starch sheath, either as the starch disappears or very shortly after its disappearance, in the root as in the radicle the appearance of the characteristic primary endodermis is *never preceded by a starch sheath*. The conception of Costantin (4, p. 47), that the endodermis might develop into

a starch sheath, is thus seen to be never in accordance with the facts. In the shoot a starch sheath appears at an early stage in development. In the light it remains, in the dark it is transformed into a primary endodermis. In the root a primary endodermis arises out of the meristem cells as they differentiate, a starch sheath is never formed.

THE NILE BLUE SULPHATE REACTION

It has been already suggested (23) that the cell walls of an etiolated shoot differ from those of a normal, light-grown shoot, mainly in the presence of more fatty substances, and during the change from a starch sheath to a primary endodermis in an etiolated stem, carbohydrates are replaced by fatty substances deposited in the Casparian strip.

Among the very many micro-chemical reagents used to investigate this change, none gave so striking a reaction as Nile blue sulphate. At the same time, to repeat the experiment successfully, it was found necessary to control the conditions with great care, and a thorough study was undertaken by Miss Sarah Ford.

Nile blue sulphate is the blue salt of a red base; with fatty acids the base forms blue salts; in neutral fats it dissolves, colouring them pink. The reaction is more marked after treatment with a 0.1 per cent. solution of sodium carbonate, and it is necessary to use the reagents in solution in glycerine, to wash and mount in pure glycerine and eliminate entirely the use of water.

Only healthy plants were used, grown under perfectly light-proof conditions, and thick sections were cut about half-an-inch to an inch below the plumular hook; these were stained for a minute in Nile blue sulphate, washed in glycerine and transferred to sodium carbonate. The sections then showed the protoplasts of the starch sheath stained a deep and intense blue in contrast to the cells of the cortex and pith which were a pale pink (Fig. 1, Plate IV). This suggests that the Nile blue base is held in the protoplasts of the starch sheath in fairly firm combination with an acid, probably a fatty acid.

When sections of a normal green plant are similarly treated there is no sign of a blue stain in the starch sheath, but the outer portion of the cortex may be stained blue (Fig. 2, Plate IV).

If the sections were washed for long with water, the reaction with etiolated plants was unsatisfactory. The reaction also failed if the sections were previously treated with alcohol or ether, so that the substance responsible for the reaction would seem to be soluble in water, alcohol and ether. When sections were previously exposed

to chloroform vapour for a moment, the staining reaction was if anything more marked, which suggests that the substance which stains diffuses slowly to the surface of the protoplast under the conditions of the experiment but diffuses more rapidly when chloroform vapour increases protoplasmic permeability.

Reactions with osmic acid strengthen the view that the Nile blue staining reaction is significant. Sections of a fresh etiolated stem, if immersed in 2 per cent. osmic acid for 24 hours, showed a distinctly stained band of cells in the region of the endodermis. No such ring could be detected in sections from a normal stem, but the outermost layers of the cortex again seemed rather blacker than those of the etiolated stem. With Flemming's solution, *sections* did not stain at all, but when pieces of etiolated stem were left in the fixative for about 24 hours, although the first few sections cut from either end were unstained, those cut from the middle of the piece were considerably blackened and the same characteristic ring of blackened cells was present around the stele. Sections previously mordanted with potassium bichromate, or chrom-acetic acid failed to stain with osmic acid. Evidently the behaviour of the pieces of stem is to be accounted for by the prior penetration of the osmic acid, probably as vapour, to the inner tissues.

Taken alone, these experiments suggest, though they do not prove, that substances of a fatty nature are being released from the cells of the starch sheath or future endodermis. The behaviour towards bichromate and osmic acid would suggest, in the light of the experience of animal histology⁽¹⁾ that these substances are rather of the nature of a phosphatide than of a true fat, but in view of the limited experience available as to the behaviour of the reagents with plant tissues, no definite conclusion can be drawn. The chemical processes involved in the change from starch grains to Casparian strip strengthen, however, the probability that fat reacting substances are present, and the change from a definitely localised reaction to a diffuse stain in the outer cortex, when the shoot is transferred to the light, is significant.

PLASMOLYSIS OF THE CORTICAL CELLS

Observations which seem of considerable interest have been made as to the behaviour of the parenchymatous cells of etiolated plants to plasmolysing agents. If sections are cut from the neighbourhood of the plumular hook of a young plant grown in complete darkness, and these sections placed in strong glycerine or 17 per cent. cane

sugar solution; no signs of plasmolysis will be seen except in the cells of the pith. If, however, the plant has been exposed to the electric lamp for two minutes each day for three successive days, then, with similar treatment, the majority of the cells of the cortex will plasmolyse freely (see Figs. 3 and 4, Plate IV). For purposes of observation the sections were usually placed for a minute in some dilute solution of a basic dye such as malachite green to facilitate the observation of the transparent protoplasts.

At first the plants thus examined were kept in the dark until cut and the sections cut under difficulty in a dull red light. Experience has shown, however, that when the plant is taken from the soil or sawdust, the sections can be cut in full light. Furthermore, prolonged exposure to light of the sections from the etiolated plant, when mounted in the glycerine, will not bring about plasmolysis.

Although two minutes illumination each day is sufficient to produce the condition necessary for plasmolysis within a short time, the effect of the light is not immediately shown in this manner. Thus completely etiolated beans were exposed to the light for three hours and sections cut directly afterwards showed no plasmolysis; but on the next day sections from others of these plants showed plasmolysis without any further exposure to the light.

A precaution that has proved necessary in the experiments is to use plants growing in pots of soil or sawdust *without transplantation*. At first beans were frequently transferred to other receptacles for the purpose of exposure, with the result that most irregular results were obtained, which were traced to the fact that seedlings so transplanted from their original place of growth often showed plasmolysis subsequently, even if kept in the dark, the phenomenon being apparently related to the disturbance of the water relation brought about by uprooting.

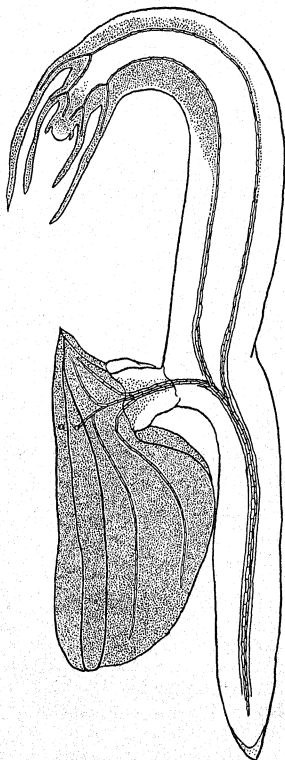
These experiments upon the plasmolysis of the cortical cells of an etiolated shoot work very uniformly now that the necessary precautions are understood. They are extremely suggestive, but in the light of our present knowledge of plasmolysis cannot be more than this. They are obviously correlated with the data provided in previous sections of the paper, and with the data as to starch distribution in the etiolated shoot which must now be briefly given.

STARCH DISTRIBUTION IN THE ETIOLATED PLANT

The distribution of starch in the etiolated broad bean and its relation to brief light exposures is worth brief record, although the

facts so far obtained only suffice to show that considerable further investigation must precede their elucidation.

Text-fig. 7 shows diagrammatically the distribution of starch in a very young seedling germinated in the dark. Starch is absent from the radicle, except in the root-cap; it is present in the cortex of the hypocotyl and in the plumule from the starch sheath up to the outermost layer of the cortex. As the plumule grows in the dark, the starch in the plumule disappears, remaining longest in the starch sheath around the stele and cortical strands but disappearing from this layer as the transformation into a primary endodermis takes place (p. 154). At the same time, in the growing region of the apex, the newly formed ground tissue, especially the cortical parenchyma, as it differentiates from the meristem is always in the first place packed with starch. It is quite easy to fail to recognise this starch in non-vacuolated, or semi-vacuolated tissues, as until the sections have been warmed in alcoholic potash and well washed, the starch reaction is masked, either completely or to a very large extent, presumably by the protein associated with it. Micro-



Text-fig. 7. Young etiolated seedling of broad bean showing starch distribution by dotting. The primary endodermis is represented by a continuous line outside the stele.

tome sections of fixed material show the distribution of the starch very clearly after such treatment. As growth proceeds, the ground tissue is again depleted of starch, which always persists longest in the starch sheaths. If now a germinating shoot is transferred from darkness to light, a very rapid disappearance of the medullary and cortical starch takes place, the disappearance always being more marked in the other tissues than in the starch sheath, in which layer it persists. If the plumule is cut off from the plant the same disappearance takes place; it is also marked in slices of the plumular hook cut and left in the light, as compared with slices from the same hook placed in the dark.

The etiolated shoots cut from the plant and placed in water lose their starch very rapidly in the light, and they also lose it, but more slowly, in the dark. If a number of plumules are sliced in half longitudinally and placed in boiled distilled water for 24 hours, then starch disappears from them, again more quickly in the light. The Maltosazone test can be obtained from the water after the necessary concentration of the solution. The same solution shows none, or the merest trace of a glucosazone. The evidence then is clear that the cells released from the root meristem do not form starch except those released from the outer surface to form the root-cap. On the other hand, the cells released from the shoot meristem uniformly produce starch, but as differentiation proceeds, accompanied with the entry of more water into the cells, the starch disappears, lingering for a long time in the starch sheath, from which it only disappears just about the time that the Casparian strip is deposited on the wall of the cells.

Zollikofer (26), in a recent investigation of the distribution of statolith starch in seedlings, which she attempted to free from starch by placing in the dark, also records the fact that starch disappears more readily from a seedling after it has been for a time in the light, than it does from a seedling which has been grown continuously in darkness.

DISCUSSION

Numerous differences between the etiolated and normal plant have been described in the previous pages. In every case this difference refers to a structural or histological feature of the plant which must be determined as a result of the behaviour of the apical meristem of the shoot. Clearly the effect of darkness upon growth has mainly to be looked for in the changes in the sequence of developmental stages by which differentiated tissues are being formed

at the growing apex. That a redistribution of the growth activities of such an apical meristem followed upon growth in darkness has already been suggested (12). The failure to release many leaf rudiments from the apical bud, the preponderance of growth in length over superficial shoot growth had already suggested that in the dark the superficial layers of meristem were making less growth, whilst new tissues were being added to the stem as the result of the activity of more deeply situated meristematic cells. Figs. 5, 6, 7 and 8, Plate V give some idea of the distribution of the meristematic cells towards the surface of the shoot in *Vicia Faba*. The greater depth of actively dividing meristematic tissues in the etiolated shoot is clearly indicated in these photographs. On the inner side also of the etiolated internode curved in the plumular hook, many of the cells around the vascular bundles are still meristematic and only just beginning to vacuolate. This difference in distribution of the meristematic tissue in the etiolated and normal plant is clearly of the first importance in determining the main morphological differences which distinguish the shoots after development.

The Plumular Hook. The plumular hook is obviously the direct result of a more rapid vacuolation and extension of the cortical cells on the outer side of the hook. This seems to be connected with the greater development of the vascular stele on this side of the stem and this in its turn is associated with the next leaf rudiment to break away from the bud, which lies on the outer and upper side of the hook. The plumular hook is then found to be associated with the alternate leaf insertions of the broad bean and pea, and the resultant unequal differentiation of the vascular stele just below the apex of the etiolated plant. The conclusion is obviously very remote from the teleological view that the plumular hook is an adaptive device to enable the shoot growing in darkness to force its way through the soil without injuring the delicate apex (5, 6 and 17).

It is, however, in better agreement with such facts as can be ascertained as to the distribution of the hook. MacDougal's comprehensive monograph (10) pays no attention to this particular feature of growth in darkness, but from his photographs and figures we learn that the following plants with opposite leaves, when grown in darkness, produce no plumular hooks, *Castanea dentata*, *Cornus alternifolia* L.f., *Hicoria minima* (Marsh) Britton, *Hicoria ovata* (Mill) Britton, *Lysimachia terrestris* (L.) B.S.P., and *Aesculus Hippocastanum* L. There are no cases described of opposite leaved plants producing a plumular hook.

On the other hand, the following plants, bearing leaves alternately when grown in darkness, in some cases from seed, in some cases when propagated vegetatively, have a plumular hook at the apex—from MacDougal(10), *Apios Apios* (L.) MacM., *Delphinium exaltatum* Ait., *Menispermum Canadense* L., *Quercus* sp., *Baccharis halimifolia* L.—from personal observation, *Vicia*, *Pisum*, *Lens esculentum* L., and *Solanum tuberosum* L.

In quite a number of cases leaves may be inserted singly at the nodes without vascular development as a consequence leading to the appearance of a plumular hook in the dark. Examples cited from MacDougal are *Aristolochia* sp. (from Bermuda), *Brassica campestris* L. (shoot from fleshy root), *Ipomoea Batatas* Poir, *Populus Simonii* Carr, and *Quercus rubra* L.

A very brief light exposure is sufficient to bring about the disappearance of the plumular hook, but as MacDougal took considerable precautions to maintain his plants in complete darkness the absence of plumular hook seen in his figures is probably significant.

When the light falls upon the plumular hook, the growing point is thrown into the erect position by the rapid vacuolation and extension of meristematic or semi-meristematic tissues on the inside of the hook. If now this plant is again transferred to the dark, as Ricôme has already reported(20), the plumular hook will re-form with the direction of the curve determined by the position of the lowest leaf still in the apical bud. This leaf will be on the outer or upper side of the hook, and when it breaks away from the bud, it moves down the stem over the outer side of the hook, then the hook will nutate very vigorously and finally turn so that the next leaf, on the opposite side of the stem, is now on the upper side of the hook.

This explanation of the appearance of the hook is complete, in so far as it relates to the morphology and anatomy of the shoot, but it requires further elucidation in reference to the factors retarding vacuolation and tissue extension on the inner side of the hook.

It was suggested in a previous paper(12) that the re-distribution of the meristem in the etiolated shoot was connected with the difficulty experienced by the meristematic cells in obtaining sufficient food for continuous growth at the surface of the shoot. This food supply reaches the meristem presumably in the form of water soluble substances, the path taken by it from vascular bundles to meristem cells being mainly, if not entirely, along the walls of the intervening

cells(18). If these walls consist of a pure carbohydrate such as cellulose, water soluble substances will diffuse in them readily, but if they are impregnated with protein or fat, diffusion will be impeded. In view of experiments in which dyes were forced into the apex under pressure, it was concluded that diffusion along these walls in the etiolated shoot was slow. Micro-chemical and macro-chemical data have since been presented(23) which indicate that the fatty substances released from the differentiating protoplasts are only slowly removed from the walls between these protoplasts, one result being the formation of a thinner cuticle in the etiolated plant(9). This slow removal of fatty substances from these walls is obviously in close correlation with the deeper seated meristematic activities. It also provides an explanation of the later appearance of a functional endodermis in the etiolated stem when fatty substances are permanently retained in the region of the Casparian strip, as a result of their oxidation whilst still contained in the wall.

If a Casparian strip is examined in sections mounted in plasmolysing solutions or such protoplasm-contracting agents as strong hydrochloric acid, the protoplast will always be seen to remain attached to this strip although contracted away from the cell wall everywhere else. This observation suggests that the protoplast is "embedded" in the substance of the Casparian strip, in which the fatty substances are oxidised. This is extremely significant in connection with the observations upon plasmolysis now recorded. The inability of the cortical cells of the etiolated plant to plasmolyse must be attributed to the fact that the surface of the protoplast is similarly embedded in the substance of the walls, and although the plasmolysing agent tends to withdraw water from the vacuole the surface of the protoplast remains firmly attached to the limiting wall. It is within a wall in this state, when in contact with the air of the intercellular spaces of the cortex, that the fatty substances undergo oxidation and thus apparently permanently embed the protoplast in this region in the substance of the wall. The protein and fat of the protoplast thus embedded in the wall in the etiolated plant, would adequately explain the slow diffusion of water-soluble nutrient materials from the vascular strands to the meristem. At the same time, the remarkable effects of short light exposure, suggest that complete vacuolation of the cortical tissues and free superficial meristem growth result from two minutes daily exposure to a very moderate artificial light. Such light exposures should free the walls from protein and fat and this observation is therefore in direct

accord with the fact that after three such daily exposures, the cortical protoplasts are readily withdrawn from the walls by plasmolysing agents.

Many recent observers suggest that the behaviour of plasmolysing protoplasts, particularly in the presence of different kations, strongly supports the inference that the substances causing adherence of the protoplast to the wall are fats or lipins or at least lipoids (Hansteen-Cranner (8), Scarth (21), Weber (24) and Weis (25)).

The behaviour of the protoplast of the endodermis, embedded in the Casparian strip, seems to supply evidence that in this early developmental stage, both the proteins and fatty substances characteristic of the young protoplast are closely intermingled with the wall. That fatty substances are a very conspicuous feature of the early stages of the development of the protoplasts of the future endodermis is certainly supported by the behaviour of these cells to osmic acid and to Nile blue sulphate.

Although then, any single item of evidence, taken alone, might be disregarded, the cumulative evidence is very strong that the change in distribution of meristematic activities at the etiolated growing point is due to the altered conditions under which food substances diffuse to the meristematic cells, as the result of the impregnation with protein and fatty substances of the walls of the cells intervening between meristem and vascular supply. Light exposures then modify the distribution of the meristem in the first place by accelerating the removal of these impregnating substances from the intervening walls. The process is accompanied by a greater movement of water and water soluble substances along these walls, one result of which is the increased vacuolation and extension of the cortical parenchyma accompanied by a rapid removal of starch from the cortical cells. The starch appears not in the most active meristematic cell, but at a very early stage and prior to vacuolation, as can be seen by the examination with iodine reagents, after previous treatment with alcoholic potash, of a microtomed section of the apex. Without this previous treatment the starch reaction of the non-vacuolated cells is masked in much the same way as the cellulose reaction of the walls of the meristematic root apex is masked by the protein with which it is impregnated (23). It would appear then that when carbohydrate supplies reach the meristem cells, which are in a condition favouring chemical condensation because they have a tendency to lose water to the adjacent vacuolated cells (11), the excess of carbohydrate may be condensed to starch, but that when water

enters the cells more freely upon exposure to light, the starch is hydrolysed to maltose.

There are at present many problems connected with starch formation and hydrolysis that await solution⁽¹⁹⁾, and in this connection these observations upon the etiolated plant are not without significance. Thus in the case of the potato plant it has always been difficult to appreciate the factors which govern the simultaneous starch hydrolysis in the leaf, and synthesis within the tuber. The present observations suggest that this problem may find a solution if the cells of the growing tuber are still unvacuolated and just passing out of the meristematic stage when the starch is deposited within them. Similarly the phenomenon of "over-growth" would become intelligible, when in a warm wet autumn after a dry summer, the apex of a fully formed tuber commences growth again. Starch hydrolysis in the cells of the adult tuber may then be associated with starch deposition in the young cells of the growing apex, near which an irregular tuber formation now takes place.

It is as yet impossible to suggest a reason for the fact that the starch is retained in the starch sheath long after it has left the rest of the tissues below the apex; in the normal plant, in light it is apparently retained in this layer, but in the dark it ultimately disappears, and at this time the Casparian strip is forming on radial and transverse walls. The network of the Casparian strip is a relatively rigid structure, resistant to stretching; its presence may in a large degree be responsible for the relatively small dimensions of the stele in the etiolated plant.

The expansion of the stele in the light-grown plant is not due to increased cell production but to greater expansion of the individual cells, and in particular to a dilatation of the pith, due to tissue tensions, in the course of which a large hollow space appears in the centre of the stem. This dilatation of the pith does not take place in the etiolated plant, possibly in part because of the resistance to stretching of the Casparian network. Other factors are, however, also at work, because at higher levels of the stem, in which an endodermis is not yet present, the etiolated stele is markedly smaller than that of the normal plant at the same level. The smaller dimension of the stele at this higher level is mainly due to the less extended condition of the medullary parenchyma. It does not seem likely, however, that this tissue fails to swell because of resistance imposed by the developing stele and cortical tissues. It is not possible to state, as a result of a preliminary comparison of serial

sections of the apices, that the procambial strands vary at all in their relative position in the cell mass in etiolated and normal plants. A comparison of the base and apex of the first internode of a normal plant, in which the base of the internode has been laid down whilst the growing point grew in darkness (beneath the soil), would suggest that the stele was laid down on a smaller radius in the tissue in the dark, as the stele and cortical strands are then more deeply sunk in the cortical parenchyma. There is, however, little difference in the number of pith cells in comparable planes of section, and as the cortex may increase considerably in number of cells at any level by a process of sliding growth, it would be unsafe to conclude that the stele differentiates in a more peripheral position when the growing point develops in the light. For the present the only safe conclusion as to the greater relative dimension of the stele in the normal plant, is to attribute it in the main to a more rapid and greater distension of the vacuolated pith cells, followed by a continual expansion of the stelar ring later, which disrupts the pith and produces a hollow stem.

In a longitudinal direction, on the other hand, undoubtedly the greater length of the etiolated internodes is due not merely to a greater extension in length of the cells, but also to an increase in numbers owing to the number of divisions of the deeply sunk meristematic cells below the etiolated apex. Brotherton and Bartlett⁽²⁾ investigated this question very thoroughly for the etiolated epicotyl of *Phaseolus multiflorus* Willd. As a result of measurements upon the epidermis of 80 etiolated and 92 normal plants they concluded that there is an increase in the number of primary meristematic divisions in darkness of 88 per cent., this accounting for 34 per cent. of the total increase of the shoot in length, the other 66 per cent. being accounted for by the increase in cell length.

SUMMARY

1. The general plan of the anatomy of the seedling of *Vicia* or *Pisum* remains unaltered by growth in darkness, but the third and further internodes usually fail to develop, the third remaining curled in the "plumular hook."

2. The diameter of the stele relatively to the stem is smaller in the etiolated plant and though considerably expanded after one hour's exposure to artificial light each day, is still slightly smaller than in the normal plant.

3. In the root the stele is limited upon differentiation by a primary endodermis, in the shoot in the dark it is bounded in the first place by a starch sheath which is later replaced by a primary endodermis. A starch sheath is never present in the roots of *Pisum* and *Vicia*.

4. A striking but fugitive staining reaction is described, given by the protoplasts of the layer limiting the stele when sections from the living etiolated plant are transferred direct to Nile blue sulphate in glycerine. From the behaviour of the layer to osmic acid reagents also, this staining reaction would appear to be due to fatty substances. The reaction disappears in stems exposed even to very brief daily light exposures.

5. It is impossible to plasmolyse the differentiated cortical cells of plants grown completely in the dark, but after very brief daily light exposures these cells plasmolyse readily.

6. The cells just below the meristematic apex are packed with starch in the etiolated shoot although the reaction with iodine reagents is not good until after treatment with alcoholic potash. This starch disappears very rapidly, except from the starch sheath, upon brief exposure to light.

7. The plumular hook is shown to be associated with the leaf habit of the plant, as leaf habit is correlated with the vascular supply and the outer side of the hook is characterised by the greater vacuolation of the cells upon the side of the stem in which the vascular elements of the stele are most fully developed. When leaves arise in pairs on opposite sides of the stem, the shoot does not form a plumular hook when grown in the dark.

8. The facts summarised above are shown to be consistent with the conclusion that the main morphological and structural features of etiolation are determined by a redistribution of meristematic growth at the shoot apex, consequent upon the greater difficulty experienced by the meristem in drawing nourishment from the vascular supply because, when growing in the dark, the walls between vascular strands and meristem are rendered relatively impermeable by the retention in them of the protein and fatty substances that form the surface of the protoplast.

The writer's grateful thanks are due to Miss Ursula Tetley for the drawings forming the Text-figs. and for the photographs reproduced on Plate V, and to Miss R. M. Tupper-Carey for the photographs of Plate IV.

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DESCRIPTION OF PLATES IV AND V

PLATE IV

- Fig. 1. Transverse section of stem of etiolated broad bean stained with Nile blue sulphate in glycerine. Stain deeply held in layer just outside stele. (\times about 33.)
- Fig. 2. Transverse section of stem of normal broad bean stained as in Fig. 1, but no signs of retention of stain in layer outside stele. (\times about 33.)
- Fig. 3. Cortical cells in a transverse section of the plumular hook of a young etiolated broad bean in plasmolysing solution, none of the cells plasmolysed. (\times 170.)
- Fig. 4. Cortical cells in a transverse section of the plumular hook of a young etiolated broad bean after exposure to artificial light for one hour on two successive days. Mounted in plasmolysing solution, all cells show plasmolysis. (\times 170.)

All photographs on this plate are of hand sections of living material, taken upon panchromatic plates with the use of colour screens.

PLATE V

- Fig. 5. L.S. of stem apex of etiolated broad bean (*Vicia Faba*) under low-power magnification, showing the general distribution of the meristematic regions. (\times about 80.)
- Fig. 6. Ditto of broad bean grown under normal conditions. (\times about 50.)
- Fig. 7. L.S. of part of stem apex of 5 under high-power magnification. Meristematic activity in the cells far below the surface of the stem apex is shown. Two stages in nuclear division can be seen to the left of cross marks. (\times 180.)
- Fig. 8. L.S. of part of stem apex of 6 under high-power magnification. Meristematic activity is here confined to the cells near the surface of the stem apex. Vacuolation is taking place in the cells which form the central core of the part of the apex shown in the photograph. (\times 160.)

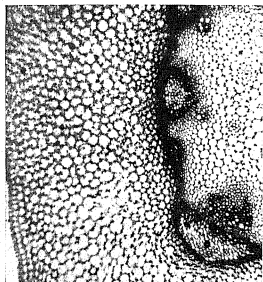


Fig. 1

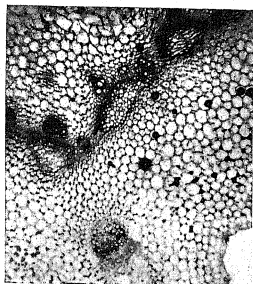


Fig. 2

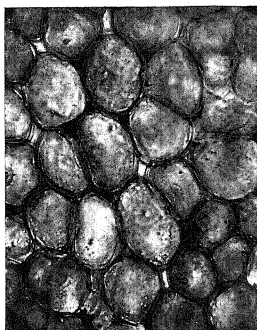
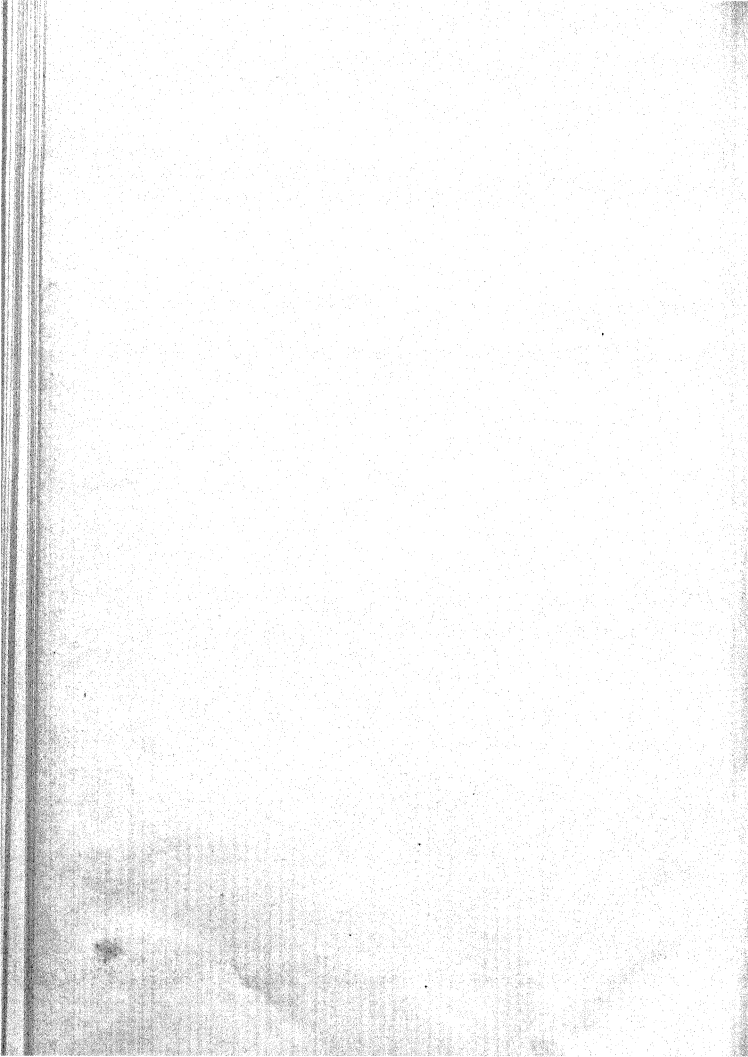


Fig. 3



Fig. 4

PRIESTLEY—LIGHT AND GROWTH



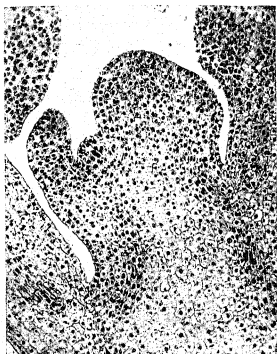


Fig. 5



Fig. 6

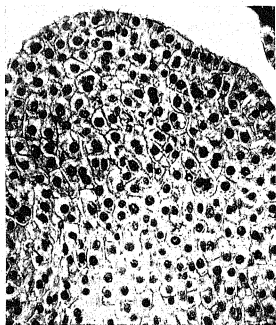
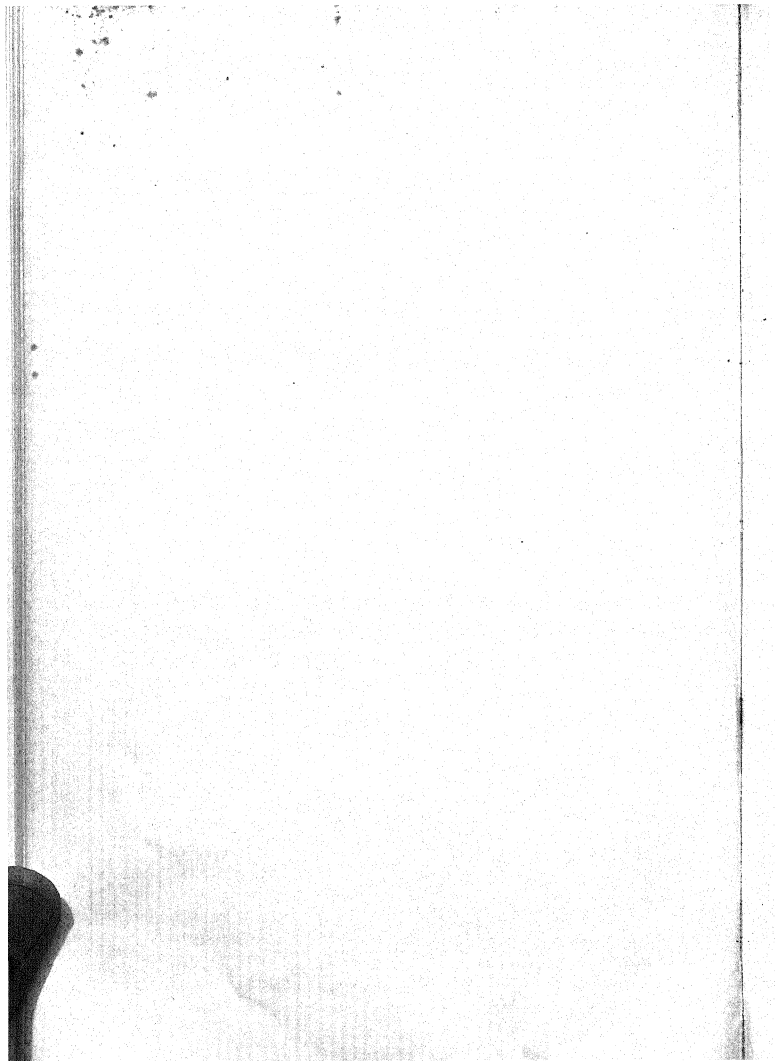


Fig. 7



Fig. 8



MYCORRHIZA

By M. C. RAYNER

CHAPTER VI

Ericaceae—Ternetz—Rayner; seed infection and the obligate relation in *Calluna*; isolation of the endophyte, and its behaviour in pure culture; shoot infection; cuttings; the cytology of digestion in the mycorrhiza cells; nitrogen fixation; nutrition—Christoph—Other members of Ericaceae—*Erica*; *Pernettya*; *Vaccinium*; *Arbutus*; *Epigaea*—Pyrolaceae.

ALTHOUGH cited by Frank as likely to exhibit specialised relations with their root-fungi, the members of Ericaceae were relatively neglected by contemporary workers at mycorrhiza, a fact possibly not unconnected with the recognition of difficulties of technique due to fineness of the roots, abundance of oil in the tissues, and comparative difficulty of manipulation under experimental conditions. Isolated observations on the mycorrhiza of *Calluna*, *Vaccinium* and *Andromeda* were recorded by Frank (1892), who described and figured infected roots, and later by Magnus (1900) and others, but no experimental work was attempted. Even in respect to root structure, the published records were singularly incomplete, confined to a limited number of genera and deficient in reliable cytological details.

The first serious contribution to the literature of mycorrhiza in the group was made by Ternetz (1907). In an earlier paper (1904), this author had published an account of a fungus isolated from a peaty soil and believed to possess the power of fixing atmospheric nitrogen. The prevalence of ericaceous species upon such soils and their invariable development of endotrophic mycorrhiza, together with the observations just mentioned, led to an attempt to isolate the root-fungi of various members of the group with a view to testing their capacity in respect to nitrogen fixation. The absence of the nitrogen-fixing bacteria, *Azotobacter* and *Clostridium*, from heath and moorland soils put on record by Beijerinck (1901) and Christensen (1906) and confirmed by Ternetz, suggested the possibility that the activities of these bacteria were replaced, in soils of this type, by those of soil fungi that might or might not exhibit symbiotic relations with the roots of the vascular plants present.

From the young roots of five ericaceous species, *Oxycoccus palustris*, *Andromeda polifolia*, *Vaccinium vitis idaea*, *Erica tetralix* and *Erica carnea*, Ternetz isolated five pycnidia-forming fungi

showing the general characteristics of the genus *Phoma*, to which were given the names *Phoma radiceis oxycocci*, *P. r. andromedae*, *P. r. vaccinii*, *P. r. tetralicis* and *P. r. ericae*. Possessing general characters in common, these forms exhibited minor morphological and physiological differences when cultured; moreover, no two of them were ever isolated from a single culture, a fact regarded as significant evidence of their specific relations in view of the circumstance that roots of two or more of the ericaceous species used for their isolation had been growing in close proximity in the field. Although the specific names just quoted implied identity with the endophytes, no proof existed that this was the case, as indeed was freely admitted by Ternetz. The necessary evidence could be supplied only by inoculations from pure cultures into fungus-free seedlings raised from sterilised seeds, with subsequent formation of mycorrhiza. Unfortunately all attempts to provide such proof were unsuccessful; in some genera, e.g. *Vaccinium* and *Andromeda*, the sterilised seeds did not germinate; in others, e.g. *Calluna*, the seedlings derived from them showed after some weeks a typically infected condition of the root-cells. Seedlings were never obtained free from mycorrhizal infection, a circumstance which suggested to the author the possibility of seed-coat infection sufficiently extensive to resist the seed sterilisation methods employed.

All the fungi isolated by Ternetz were tested for nitrogen-fixation by cultivation in liquid media free from combined nitrogen, aerated by means of a slow stream of purified air passed through the culture flasks. After four weeks' growth, the nitrogen contents of mycelia and culture fluids were estimated separately by the Kjeldahl method. All the cultures yielded evidence of nitrogen-fixation, although in very different degree. The fungi extracted from *Oxycoccus*, *Vaccinium* and *Andromeda* showed the greatest activity, the values recorded after four weeks' growth being respectively 18 milligrams, 22 milligrams and 11 (10.92) milligrams of nitrogen assimilated for each gram of dextrose used.

As compared with the values obtained by Winogradsky (1902) for *Clostridium pasteurianum* and Gerlach (1902) for *Azotobacter chroococcum*, the absolute amounts of nitrogen fixed by these fungi are relatively insignificant. On the other hand, they worked far more economically than did the bacteria in relation to the amount of sugar consumed during growth, and in this respect were the most efficient nitrogen-fixers yet recorded. For purposes of comparison, Ternetz tested the possibility of nitrogen-fixation by *Aspergillus*

niger and *Penicillium glaucum* and obtained evidence of a relatively feeble capacity to utilise gaseous nitrogen on the part of these species also, a result subsequently corroborated by Froelich (1907). The comparative values obtained for all the fungi named and also for *Clostridium* and *Azotobacter* are set out in the table now reproduced (Table I).

TABLE I.

(From Ternetz, *Jahr. f. wiss. Bot.* Bd. XLV, p. 388, 1907.)

Name des assimilierenden Organismus	Dauer des Versuches Tage	Gebotene Dextrose		Verarbeit. Dextr. gm.	N-Ge-winn mg.	Ass. N pro 1 gm. verarb. Dextr. mg.	Bemerkungen
		gm.	%				
<i>Clostridium Pasteurianum</i> (Winogradsky)	20	40	4	40	53.6	1.34	
<i>Clostridium Pasteurianum</i> (Winogradsky)	20	20	2	20	24.4	1.22	
<i>Clostridium Americanum</i> (Pringsheim)	30	1.25	0.25	1.25	4.6	3.7	
<i>Clostridium Americanum</i> (Pringsheim)	30	5	1	3.01	8.2	3.01	
<i>Azotobacter chroococcum</i> (Gerlach und Vogel)	35	5	0.5	5	42.7	8.56	Mittel aus 2 Besten
<i>Azotobacter chroococcum</i> (Gerlach und Vogel)	35	12	1.2	12	127.9	10.66	
<i>Aspergillus niger</i> (Ternetz)	28	7	7	1.1	1.9	1.71	
<i>Penicillium glaucum</i> (Ternetz)	28	7	7	0.7	2.8	3.8	
<i>Phoma rad. Oxycocci</i> (Ternetz)	28	7	7	0.85	15.3	18.08	
<i>Phoma rad. Andromedae</i> (Ternetz)	28	7	7	0.67	7.3	10.92	
<i>Phoma rad. Vaccinii</i> (Ternetz)	28	7	7	0.71	15.7	22.14	nicht ganz bakterienfrei
<i>Phoma rad. Tetralicis</i> (Ternetz)	28	7	7	1	4	3.99	
<i>Phoma rad. Ericae</i> (Ternetz)	28	7	7	1.1	2.3	2.17	

Comparative cultures of the different forms showed that the amount of nitrogen fixed was independent of increase in dry weight, the two forms of *Phoma* which showed the greatest increase in dry weight after four weeks' growth giving the smallest values for assimilation of nitrogen. Of the latter, the greater part was contained in the nutrient fluid in each culture, only a small proportion being found in the mycelium. It is significant also that in no case was a supply of combined nitrogen required for active growth of the cultures.

Due precautions were observed by Ternetz in respect to technique and the adequate provision of check and control cultures, and the estimations were made with great care. Some doubt was expressed

by the author respecting the adequacy of the Kjeldahl method for dealing with very small quantities of nitrogen, but it was concluded on the basis of an independent series of tests, that the method was satisfactory if the values actually obtained were treated as approximate only. The absence of any grounds for adverse criticism of Ternetz' experiments is endorsed by Duggar (1916) in a critical review of work dealing with the vexed question of nitrogen-fixation by fungi.

The path of the experimental worker who attempts to prove the assimilation of atmospheric nitrogen by micro-organisms is a particularly thorny one. The values obtained are of necessity relatively minute, and the possibilities of experimental error relatively great. Moreover, in the case of soil organisms, the matter is of great practical importance and very rigid proof is rightly demanded before the claims made can be regarded as definitely established. Recognition of the value of Ternetz' work on nitrogen assimilation by the endophytes of Ericaceae has been unduly delayed owing largely to the author's failure to establish the identity of the fungi isolated. Subsequent work has provided ample confirmation of her conviction that she was working with the true endophytes, and rendered possible a belated tribute to the skilful and accurate technique by which their isolation from the roots was successfully accomplished. It has also furnished corroborative evidence that the root-fungi of ericaceous species can assimilate atmospheric nitrogen.

Exact information respecting the bionomics of mycorrhiza in Ericaceae dates from the publication of a paper on *Calluna vulgaris* (Rayner, 1915).

Experimental work was initiated by a study of the edaphic relations of this species in a selected area, and an attempt to evaluate the significance of the calcifuge habit (Rayner, 1911). Field observations indicated that the failure of the plant to spread by seed in the area investigated was apparently strictly correlated with a rise in the calcium carbonate content of the soil, with which was associated a shifting of the reaction in the alkaline direction. Experiments were devised to investigate and analyse the soil factors concerned in this limitation of growth with the view of throwing light on the significance of the calcifuge habit in the group as a whole. Pot cultures in calcareous and non-calcareous soils yielded evidence of abnormal growth in the former as evidenced by reduced germination capacity, retarded rate of germination, arrest of root growth and distorted development of the growing region, and arrest of shoot

growth with reduced size and red coloration of the foliage leaves. Intimately associated with the abnormalities of growth noted in plants grown in calcareous soils was the presence of dense colonies of bacteria on the surface of the roots, especially around the tips, and a marked diminution of vigour in the growth of the mycorrhizal fungus normally present. It was found, moreover, that similar growth reactions could be induced in seedlings growing in a favourable soil by watering with filtered extracts of calcareous soil.

At this stage of the investigation it was not clear whether the bacteria present upon the roots in calcareous soils were pathogenic agents directly responsible for the abnormal behaviour of seedlings, or were to be regarded merely as indicators of soil conditions unfavourable to growth and, incidentally, to the maintenance of the favourable relations maintained with the mycorrhizal fungus under favourable soil conditions. Subsequent work with soil extracts under "pure culture" conditions proved the correctness of the second view (Rayner, 1920).

Laboratory experiments pointed to root-infection as an important biological factor controlling growth, and directed attention to the study of its origin and significance as an initial step towards an understanding of the somewhat specialised soil relations shown by *Calluna* and allied species (Rayner, 1913). Before, therefore, any specific edaphic problem involving soil preference could be attacked directly—before, indeed, such a problem could be clearly formulated—it was essential to prepare the way by undertaking an independent investigation into the physiology of the relationship between the *Calluna* plant and its mycorrhizal fungus. The difficulties and problems faced by Frank and all subsequent workers upon the biology of mycorrhiza immediately presented themselves. At what stage of development and from what source does root-infection take place? Is it possible to cultivate seedlings free from fungus infection? Can the root-fungus be isolated; how does it behave in pure culture; and what are its systematic affinities?

In the case of *Calluna* experimental research has provided answers to these questions and disclosed the existence of a remarkable and unsuspected biological relation between the green plant and its fungal associate, a relation resembling that found in Orchids in that it involves dependence of the seedling plant upon infection by an endophyte at a critical stage of development, but differing sharply therefrom in respect to the mode of infection and the widespread extension of mycelium throughout the shoot tissues (Rayner, 1913,

1915). As in Orchids, there is an obligate relation between the two organisms, and proper development of the seedling is bound up with infection by the appropriate fungus at germination. In Orchids, such infection is precarious, depending upon the presence of the root-fungus in roots of plants of the same species or in the soil around them in the proximity of germinating seeds; in *Calluna* infection is ensured by the presence of mycelium of the appropriate fungus upon the testa of the ripe seed. In Orchids, the distribution of the endophyte within the plant body is strictly limited; in *Calluna*, it is almost co-extensive with the tissues of the plant; mycelium extends throughout the root and shoot, spreads into the floral organs and passes from the columella and walls of the fruit to the testa of the developing seed. Only the resting embryo and endosperm are free from fungal infection; at all other stages of development and throughout its active vegetative existence every *Calluna* plant yet examined has been found to be a dual organism.

The full story of the association with the mycorrhizal fungus was pieced together as a result of experiments extending over a number of years. For a complete account of the researches, reference must be made to the papers cited and to those published subsequently (Rayner, 1921, 1922, 1925). The main facts are included in the following summary: The young roots of *Calluna* are extremely fine, consisting only of an axial vascular strand surrounded by a single layer of large cortical cells. Under normal conditions of growth each of the latter encloses a dense branch system of mycelium, the whole root forming a characteristic endotrophic mycorrhiza. Each intracellular hyphal complex is continuous with hyphae upon the external surface of the roots, some fine and hyaline, others brownish and of large diameter. The intracellular hyphae are of relatively large and uniform diameter with abundant oily contents, the latter being absent from the surrounding cytoplasm (Pl. VII, Figs. 35, 36). The mycelium of the endophyte has not been observed to penetrate within the vascular cylinder of the root.

Throughout the growing season the mycorrhiza cells exhibit active intracellular digestion of mycelium with disappearance of the resulting—and presumably soluble—products. The nearer to the apical meristem, the more rapidly is digestion initiated. Its onset is marked by the usual signs of cell activity—increase in size and chromatic content of the nuclei often accompanied by deformation, “clumping” of the mycelium about the nucleus, disappearance of the sharp outlines of individual hyphae, and the gradual conversion

of the hyphal constituents from the region of the nucleus outwards, to a structureless mass possessing strong stainability. The last stages in the process are marked by shrinkage of the nuclei and disappearance of the stainable contents (Pl. VII, Fig. 37). This intracellular digestion of mycelium is a continuous process observable throughout the vegetative season from early spring to late autumn. The proportion of cells in the active mycelial condition or undergoing digestion at any given moment varies with the time of year, the age of the root, and possibly also with the season and other external factors. The phenomenon of digestion was overlooked by most of the earlier observers, although Frank (1891) referred to the infected cells in roots of *Ledum* and *Empetrum* as showing similar staining reactions to those of Orchids, and (1892) figured a cell from the mycorrhiza of *Empetrum* showing what was probably the "clump" stage of digestion. Magnus (1900), fresh from observations on the Orchids, stated that only "Pilzwirthezellen" were present in the mycorrhiza of *Calluna*. Since the formation of mycorrhiza in *Calluna* is an annual phenomenon, the ultimate fate of the constituent cells is of little physiological importance; in roots which persist for a second season, they are exfoliated by the formation of pericyclic cork.

Certain recent observations on the formation of mycorrhiza by *Calluna* have an important bearing on the nature of the biological relation between the two organisms concerned, and incidentally explain the inability to observe root-infection recorded by certain workers. During a seasonal study of the mycorrhiza of *Calluna*, it was observed that the early spring roots were characterised by rapid growth and much lighter infection than was the case later in the season. This relative immunity to infection is believed to depend on the interaction of two sets of causes: (a) the differential effect of low soil temperatures on the growth of root-cells and mycelium respectively; (b) the existence of internal factors regulating the reaction of the cortical cells to infection, related in turn with certain external conditions controlling the rate of growth.

Partial immunity to infection, with a similar imperfect or abortive formation of mycorrhiza, was observed subsequently in healthy plants subjected to certain experimental conditions; for example, cultivation in a favourable soil or soil extract sterilised by heat, in sterilised sand irrigated with rain water or a weak solution of salts, and in agar nutrient. The conclusions drawn from these observations were thus summarised: "*The development of the endophyte in the mycorrhiza cells of Calluna is markedly inhibited by certain conditions*

of the rooting medium, and roots exposed to such conditions may appear to be uninfected" (Pl. VII, Fig. 40). It may reasonably be inferred that the formation of active mycorrhiza in *Calluna* is a "reciprocal phenomenon," conditioned not only by the activity of the fungus, but by the reaction of the root-cells to invasion, and by factors in the rooting medium directly related with the nutrition of either partner, or both (Rayner, 1925). One other conclusion stated in the same paper may be quoted: namely, that "fungal infection and the stimulus to development associated with it on the one hand, and the formation of root mycorrhiza on the other hand, must be regarded as distinct phenomena." There is no experimental evidence that the formation of mycorrhiza is obligate, although under ordinary soil conditions it is the natural sequence to seedling infection and may be closely bound up with the soil relations of the roots.

The possibility or otherwise of replacing the stimulus to development ordinarily supplied by infection by the addition of a suitable organic substance to the rooting medium has not yet been fully explored in Ericaceae. In view of the asymbiotic germination of Orchid seeds when supplied with appropriate organic material, it seems reasonable to infer that similar methods might be used successfully in Ericaceae, and that seedlings thus raised free from infection might grow satisfactorily without mycorrhiza. On the other hand, further experimental data are required respecting the length of time during which plants of various species of Orchid can be maintained in healthy growth lacking infection; the recent work of Huber (1921), on *Liparis loeselii*, has yielded results at variance with those of other workers and is possibly significant in this connection (see p. 103). This matter will be discussed more fully in a later section dealing with the physiology of nutrition.

The identification of mycelium in the shoot tissues of *Calluna* is a matter of great difficulty and can be accomplished only with the aid of a delicate and specialised technique. In the young seedling immediately subsequent to infection it is relatively easy to trace the path of the invading hyphae and occasionally to observe an intracellular complex resembling those in the root-cells in the leaf mesophyll. Such intracellular mycelium is subject to eventual digestion by the host cell. In general, the hyphae which traverse the stem tissues are excessively fine and restricted in position to the middle lamellae of the cell-walls. Otherwise, they show no special distribution but range throughout all tissues, vascular and otherwise (Pl. VII, Fig. 38). Only in air spaces in the leaves and elsewhere,

and in the dead tissues of the bark, are hyphae of normal size and structure to be found. The extension of mycelium throughout the shoot was experimentally verified by rooting cuttings under controlled conditions (Rayner, 1925). It was found that shoot cuttings, rooted under aseptic conditions in sterilised sand, formed a normal root system with infection of the type characteristic for plants growing in a sterilised rooting medium. Moreover, the endophyte has been extracted from similar shoot cuttings. These experiments with cuttings are believed to constitute a final reply to the claims put forward by Christoph (1921) in respect to non-infection of *Calluna* (see p. 181). (Fig. 29 and Pl. VII, Figs. 39, 40.)

In the organs of the flower mycelium is abundant, and much of it undoubtedly belongs to the endophyte. In the tissues of the ovary wall, as in the vegetative axis, the hyphae are extremely fine and can be identified with difficulty; thence they spread to the outer cell layer of the seed-coats, a phase of infection possibly related to the breakdown of these cells in the final stages of seed development and one not difficult to observe in fortunate sections (Rayner, 1925, Plate VI, Fig. 1 a). Ripe seeds removed from unopened fruits carry fine mycelium, sometimes profuse, more often extremely scanty and difficult to put in evidence (Pl. VI, Fig. 32). At germination this mycelium becomes active and infection of the seedling root by fine hyphae can readily be observed in seeds germinating on filter paper. A similar infection stage has been noted by the writer in seedlings of *Pernettya* which had germinated viviparously within the fruit chamber, so repeating the observation made by Ternetz (1917) on *Andromeda polifolia*.

By careful methods of sterilisation it is possible to kill the mycelium of the endophyte upon the testa as well as free the seeds from casual contamination by micro-organisms. Seeds, so treated, germinate normally under aseptic conditions, but the resulting seedlings exhibit more or less complete arrest of shoot development with an inhibition of root formation which is practically complete.



Fig. 29. *Calluna vulgaris*: cutting rooted in sterile sand under controlled conditions. Removed and photographed 40 days after insertion in tube. S, stem.

If inoculated at planting from a pure culture of the endophyte, they develop normally (Pl. VI, Figs. 30, 31).

The endophyte has been isolated from young roots, from shoots, and repeatedly from seeds removed aseptically from unopened fruits. It is a pycnidia-forming fungus with the characters described by Ternetz for *Phoma radidis*, and the name *P. r. callunae* given to the form isolated by that author has therefore been accepted. The necessary proof of identity which Ternetz failed to obtain has been provided by re-inoculation into seedlings free from infection, and the identity of the forms isolated by that worker with the endophytes of a number of ericaceous species may be regarded, therefore, as finally established.

A sporing colony of the endophyte in pure culture is shown in Plate VII; for the characters of the mycelium and pycnidia reference may be made to the original description and figures (Rayner, 1915, Plate VI, Figs. 8, 9). In old cultures the mycelium produces terminal and intercalary swellings which have also been observed in association with roots in nature. It can be cultivated on many different artificial media over a considerable range of pH values, but is liable to show marked change of behaviour, e.g. in relation to pycnidia formation, under prolonged cultivation on artificial nutrients. Its physiological properties are easily altered by changes in the substratum; in particular the power of promoting development in the seedling is readily impaired both by long cultivation outside the plant and by the nature of the food supplies. In general, such change is in the direction of active parasitism. (Pl. VII, Figs. 41, 42.)

The evidence available bearing upon the physiology of nutrition will be discussed in a later section of the present work.

In view of Ternetz' researches on nitrogen assimilation by a number of forms of *Phoma radidis*, the reaction of the *Calluna* plant to substrata free from combined nitrogen is of special significance. Researches on this subject (Rayner, 1922) showed that "pure culture" seedlings, inoculated with the endophyte at planting and grown in an agar medium lacking combined nitrogen, thrive as well as did the control seedlings to which potassium nitrate was supplied at the rate of 0.5 g. per litre. The seedlings not supplied with nitrate were, on the average, healthier than the controls, of bright green colour and of equally vigorous growth. Kjeldahl estimations of samples of the agar medium used for these cultures yielded negative results, and the conclusions were confirmed by subsequent work using a silica jelly substratum instead of agar agar.

Indirect evidence of nitrogen-fixation by members of the genus *Phoma* has also been supplied by Duggar and Davis (1916) in the course of a critical experimental study of the evidence for nitrogen-fixation by fungi, these authors having adopted special precautions to avoid experimental methods open to criticism on the score of inaccuracy. Among the species investigated were *Penicillium* spp., *Aspergillus niger* and *Phoma betae*. With regard to the two first-named genera, Duggar confirmed the experimental conclusions of Ternetz and others that these fungi can utilise atmospheric nitrogen to a very slight extent. In the case of *Phoma betae*, the values obtained ranged from 3.022 to 7.752 mg. per 50 mg. of culture fluid in 25 days, a known amount of combined nitrogen being supplied. These values are outside the range of experimental error and are of special interest for comparison with those obtained by Ternetz for the forms of *Phoma radialis*.

Differences of structure and behaviour were noted by Ternetz in the forms of *Phoma* isolated from different ericaceous species. This evidence of specificity in the endophyte is supported by the observed impracticability of using the *Calluna* endophyte for promoting germination in other genera, for example, in *Erica tetralix*, *Erica cinerea* or *Pernettya mucronata*.

Mention must here be made of the discordant experimental results reported subsequently by Christoph (1921) regarding fungus infection in members of Ericaceae. In respect to *Calluna vulgaris* and *Erica carnea*, it has been put on record by this observer that seedlings both from sterilised and unsterilised seeds remained free from fungus infection when grown on thoroughly sterilised soil from a *Calluna* station, while similar seedlings grown on the same soil untreated rapidly developed mycorrhiza of the characteristic type.

From the time of Frank onwards, the members of Ericaceae have been regarded as typical examples of obligate mycotrophy. Christoph rejects this view and reports that in very dry places, plants of *Calluna vulgaris* and *Erica carnea* are often found lacking mycorrhizal infection altogether, while in pot cultures allowed to become dry the fungus soon disappears. In respect to this, it was not stated whether plants were kept under observation during the whole of the growing season. Moreover, by rooting cuttings in sterilised soil, he obtained plants that were believed to show complete freedom from root-infection. It was inferred by Christoph that infection took place only from the soil, thus precluding the possibility of seed-coat infection.

Unfortunately, no satisfactory evidence has been provided that the seeds used for these experiments had been effectively sterilised, nor has adequate proof been supplied that a fungus isolated from *Calluna* roots was the true endophyte. On the other hand, it is believed by the present writer that the supposed absence of infection from roots of seedlings and cuttings grown in sterilised substrata, and from plants occurring in nature in dry situations, is adequately explained by the partial suppression of mycorrhiza formation under these conditions. Infection takes place inevitably—in the case of seedlings, from the seed-coats, in the case of cuttings, from the stem tissues—but the normal mycorrhizal condition is not established under certain well-defined rooting conditions. The invariable presence of mycelium in the root-cells and elsewhere in such plants can be demonstrated by the aid of a careful technique (cf. Fig. 40).

It is clear that, in nature, ericaceous seedlings germinating in the neighbourhood of the parent plants will always be liable to infection from the soil in addition to that derived from mycelium upon the seed-coats. A detailed criticism of Christoph's work will be found in two papers by the present writer (Rayner, 1922, 1925).

Infection of the ovary tissues by mycelium has been recorded for a number of other ericaceous species of widely separated affinities (Rayner, 1915). It seems likely, therefore, that the habit described for *Calluna* is common in the family Ericaceae, although it may be manifested in different forms and the degree of dependence of seedling development upon infection may vary within wide limits. Experimental evidence has already been secured that in certain species, e.g. *Erica cinerea*, *Erica tetralix* and *Pernettya mucronata*, development of the seedling, lacking infection, progresses no further than in *Calluna*. On the other hand, it has been stated recently by Melin (1921) that the seeds of a race of *Azalea mollis* investigated by him are free from mycelium. This, if correct, should involve a capacity for independent development in the resulting seedlings.

For various reasons the genus *Vaccinium* is of rather special interest. The systematic affinities of the Vaccinioideae are somewhat uncertain, while the edible fruits produced by many species of *Vaccinium* constitute a claim to interest on the economic side; moreover, it was specifically stated by Stahl (1900) that roots of *Vaccinium myrtillus* remained entirely free from fungus infection when plants were raised from untreated seeds in sterilised soil, and he added to this record the following generalisation:—"Während manche obligaten Mycorrhizenpflanzen, wie wir früher gesehen

haben, der Anzucht aus Samen und der Kultur grosse Schwierigkeiten bereiten, lassen sich die Ericaceen auch ohne Gegenwart von Wurzelpilzen unschwer kultivieren, und ihre Samen gehen, zwar oft langsam, aber in grossem Procentsatz und sicher ohne Mitwirkung symbiotischer Pilze auf." Since the roots of *Vaccinium myrtillus* under normal soil conditions possess well-developed mycorrhiza of the usual ericoid type, this statement, if correct, precludes the possibility of infection from mycelium present on the seed-coat, and involves direct infection from the soil in the manner believed at one time to be invariable in mycorrhiza plants.

With the view of elucidating the exact condition in the genus, a series of experimental researches on species of *Vaccinium* have been carried out by the writer subsequent to the year 1915. The species used for the greater part of the work were *Vaccinium oxycoccus* (*Oxycoccus palustris*) and *Vaccinium macrocarpum*. The results of these researches will shortly be published. They may be anticipated here by two statements bearing directly on the claim put forward by Stahl in the passage just quoted. It has been found that: (1) seeds of *Vaccinium oxycoccus* and *Vaccinium macrocarpum* are infected by their respective endophytes while still contained in the fruit, and (2) infection of the seed-coat of these species of *Vaccinium* is more deep-seated than in *Calluna*. These observations render it difficult to accept Stahl's statement that plants of a closely allied species of *Vaccinium* were "völlig pilzfrei" after five months' growth in sterilised soil (see p. 69). The fact of root-infection in *Vaccinium* was probably overlooked by Stahl, as more recently by Christoph in *Calluna*, because the formation of mycorrhiza is partially inhibited in the roots of both species when growing in a sterilised medium. Under these conditions the demonstration of mycelium in the mycorrhiza cells of the roots demands a more careful technique than was bestowed upon it by either of these observers.

Before leaving the subject of *Vaccinium*, the work of Coville on the Blueberry, *Vaccinium corymbosum*, may be mentioned (Coville, 1910, 1916, 1921). In an account of this species, cultivated for the sake of its berries on wet peat soils in the United States, Coville described and figured the mycorrhiza cells of the root but did not carry out experimental researches on the subject of infection. On general grounds of habitat and the known shortage of available nitrogen compounds in peat bogs, he favoured the view that this species of *Vaccinium* can indirectly utilise gaseous nitrogen through the intervention of its mycorrhizal fungus. The positive results

obtained by Ternetz for the endophytes of other species of *Vaccinium* were cited by Coville in support of this opinion.

Only one other member of the Ericaceae, viz. *Arbutus unedo*, the Strawberry Tree, has been the subject of experimental investigation in respect to mycorrhiza. Whereas, in the case of most members of the group, the external structure of the roots is ordinarily unaffected by the development of the endotrophic mycorrhiza, many of the lateral roots of *Arbutus* develop into small tubercles (Dufrénoy, 1917). Dufrénoy's material was obtained from plants growing wild in certain districts of south-western France, where *Arbutus* has a curiously limited distribution in the old Pine forests of Arcachon and La Teste but does not extend into the neighbouring Pine woods of Gascony.

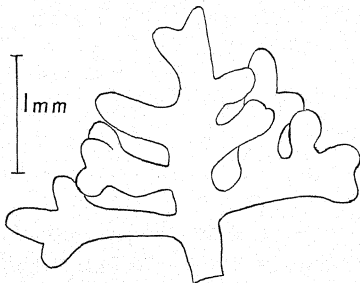


Fig. 43. *Arbutus unedo*: old branched tubercle. (From Rivett, *Annals of Botany*, 1924.)

The structure of the mycorrhiza in this plant has recently been re-investigated more fully by Rivett (1924), using material from seedlings raised in the British Isles. Rivett describes two distinct types of root in *Arbutus*, long roots which form the main root system, and root tubercles, shown by the author to be arrested laterals and sub-laterals of the season's growth (Figs. 43, 44). The long roots show a superficial network of mycelium with occasional penetration by hyphae; the type of infection bears some resemblance to the typical mycorrhiza of other members of Ericaceae but the intracellular development of the endophyte is relatively slight. The tubercles may be simple or branched, and are described as "relatively impermanent" organs. They originate from young lateral roots which suffer arrest of growth owing to profuse infection of the

ectotrophic type. Subsequently, extensive endotrophic infection of the peripheral tissues occurs with rapid and complete digestion of the intracellular hyphae. Marked differences in the character of the mycelial growth, associated with the long roots and tubercles respectively, are described by the author who considers that the growth of the mycelium associated with roots is influenced by the presence of much mucilage on the emerging laterals, and by excretions from the ruptured tissues of the parent root. The presence of a mucilaginous sheath over the surface of the young roots is held by Rivett to be a normal feature of the root system in *Arbutus*, in disagreement with the view expressed earlier by Dufrénoy, who regarded it as a secondary feature associated with the presence of bacteria and algae. The mucilage is very variable in degree of development and may become conspicuous under dry rooting conditions. The roots of *Calluna* show a similar condition, the mucilage exhibiting precisely similar reactions to those described for *Arbutus*. In the former plant also it is abundantly developed in the neighbourhood of emerging laterals and around the young root-tips. In *Calluna*, as in *Arbutus*, it may become conspicuous when the rooting conditions are dry and may then serve to protect the young roots from drought. In both it is often well developed in perfectly clean young roots and doubtless plays a part in casual epiphytic infection by micro-organisms, perhaps also exerting a chemotactic influence on the initial infection of the seedling root by the mycelium of the endophyte in *Calluna*. (Pl. VI, Fig. 33.)

A curious feature of these tuberous mycorrhizas of *Arbutus* is the development by some of the hyphae in the outer part of the fungal sheath of stiff bristle-like setae up to 0.1 mm. in length. They occur only on young tubercles and on superficial examination resemble root-hairs (Fig. 45).

No information respecting the behaviour of uninfected seedlings of *Arbutus* is at present available. Ovarial infection has been recorded for the species (Rayner, 1915). Whether this, as in *Calluna*, carries with it a similar mode of infection at germination, and whether seedling development is bound up with such infection, are questions awaiting experimental solution. In view of the marked difference of structure and distribution of mycelium described it is tempting to speculate on the possible association of more than one fungus species with the roots of *Arbutus*. The presence of mycelium of more than one kind is not suggested by Rivett and must remain a matter of speculation until the isolation and cultivation of the

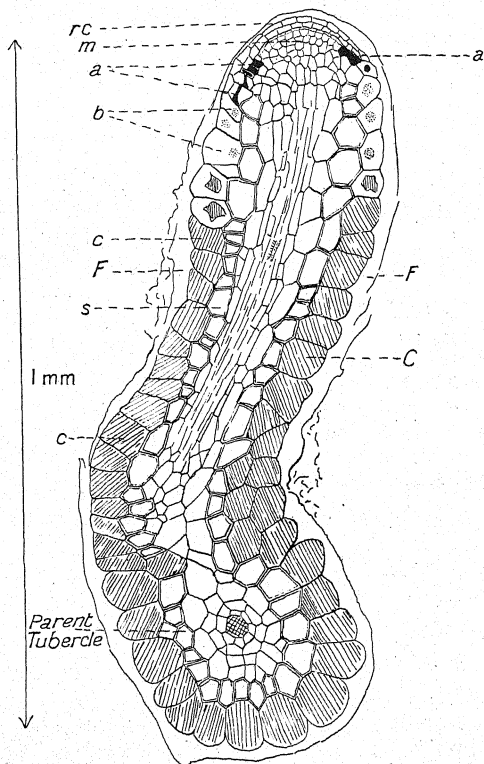


Fig. 44. *Arbutus unedo*: longitudinal section of young tuber; rc, root-cap; m, meristem; a, first infected cells; b, cell with contents completely digested and large granular nucleus; c, reinfecting cells; F, external hyphae; s, suberised layer. (From Rivett, *Annals of Botany*, 1924)

mycelium associated with the roots has been successfully accomplished.

Although *Arbutus* is exceptional in producing tubercles, the capacity for doing so exists in other species, e.g. in *Calluna vulgaris*.

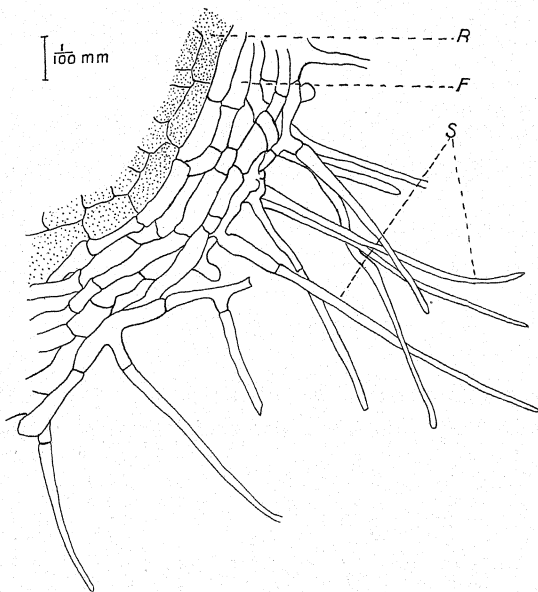


Fig. 45. *Arbutus unedo*: young tubercle with setae; S, setae; F, exterior fungal hyphae; R, root-cap cells of host. (From Rivett, *Annals of Botany*, 1924.)

The photograph on Pl. VI, Fig. 34 shows the formation of root-tubercles by a healthy plant of Ling from a typical callunetum in the south-east of England. The plant was vigorous and the roots showed no signs of pathogenic infection. The detailed structure of these tubercles has not yet been described.

Councilman (1923) has recently recorded the formation of mycorrhiza by *Epigaea repens* in America. Herbarium material from northern Japan showed an identical condition, a fact used by the author as an argument that the relation prevailed in the plants at their common source before the last glacial epoch. It was observed that the mycorrhiza cells sometimes contained material derived from alteration of the enclosed mycelium, and that such cells might undergo fresh infection. Presumably, therefore, intracellular digestion, of a similar nature to that described for *Calluna*, takes place regularly in *Epigaea*.

The mycorrhiza of *Epigaea* has not been the subject of experimental research, and Councilman has not at present correlated his observations with the results of experimental enquiry on *Calluna* and other members of Ericaceae.

For the smaller groups included in the Ericales, the available data respecting root infection and the biology of mycorrhiza where it occurs, relate almost exclusively to the Pyrolaceae. The members of this family are grouped in two sub-orders of which one, Pyroloideae, contains the chlorophyllous genera *Pyrola* and *Chimaphila*; the other, the Monotropeideae, includes *Monotropa* and the allied non-chlorophyllous genera usually described as holosaprophytes¹.

Mycorrhiza is formed by all members of the Pyrolaceae, and it has been pointed out by Henderson (1919) that it is possible to trace a series among the green forms showing a gradual transition from species with a relatively feeble production of mycorrhiza, e.g. *Chimaphila umbellata*, to others with heavier infection, e.g. *C. maculata*. In certain species of *Pyrola* some epidermal cells only are infected; in others, all the epidermal cells of the roots are filled with mycelium, there are indications of the formation of a fungal sheath about the root-tip, and rhizomes as well as roots are heavily infected. In the Monotropeideae, chlorophyll is not formed, and there is a conspicuous fungal sheath about the roots showing differentiation into two zones.

In the paper to which reference has already been made, Christoph (1921) has given an account of his observations on members of the Pyrolaceae. Working with *Pyrola uniflora*, *P. secunda*, *P. minor*

¹ The groups included by Engler in Ericales are as follows:—Clethraceae, Pyrolaceae, Lennoaceae, Ericaceae, Epacridaceae, Diapensiaceae. In other classifications the Vaccinioideae, a sub-order of Ericaceae, is treated as a separate family, Vacciniaceae, the genera *Pyrola* and *Chimaphila* are placed in Ericaceae, and the non-chlorophyllous members of Pyrolaceae (Monotropeideae) separated as a distinct family, Monotropaceae.

and *P. rotundifolia*, he reached the conclusion that, as in Ericaceae, no true symbiosis existed. Nor was he able to find any direct correlation between fungus infection and seedling development, or between infection and the formation of "coralloid" roots. Root-infection was described as involving an intercellular development of mycelium, but in *Pyrola* a typical ectotrophic structure was found only in those species which possessed rhizomes and only when growing in soils rich in humus.

The seeds of *Pyrola rotundifolia*, a species showing well-developed mycorrhiza in certain localities, germinated independently of infection, and the same was believed to be true for other species of *Pyrola* observed to germinate naturally on dry soil from which the fungus was judged to be absent. Seeds of the first-named species did not germinate upon sterilised soil, but success was achieved by the addition to the substratum of various organic substances, e.g. concentrated soil extract and peptone solution, the best results being obtained by supplying a mixture of these two substances. The effect of the root-fungus upon germination was apparently not tested.

It was believed that all species of *Pyrola* are liable to infection by closely related fungi, the mycelium of which showed "clamp connections." The mycelium associated with members of the Monotropoideae has no "clamp connections" and presumably, therefore, does not belong to members of the Basidiomycètes.

In respect to the biology of fungus infection in Ericales, Christoph concluded that, except in the Monotropoideae, there is no evidence of exchange of nutritive material; in all other cases, the endophyte is a harmless parasite.

In view of the predilection shown by many members of the Pyrolaceae for humus soils and their association with non-chlorophyllous species, the results recorded by Christoph are somewhat unexpected. Members of the family have so many features in common with Ericaceae and Orchidaceae, e.g. the possession of small seeds and undifferentiated embryos, and a marked susceptibility to fungus infection, that it would not have occasioned surprise to find a similar dependence of seedling development upon invasion by the root-fungus. Discussing the germination of "dicotylous saprophytes" in general, Goebel (1905) observed that it apparently took place only in very special surroundings:—"Probably the fungi which are found in the roots in symbiosis are essential."

The family Epacridaceae was one of those cited by Frank as likely to show specialised relations with root-fungi. Alike in this

group, in Diapensiaceae, and in Clethraceae, the mycorrhizal relations require investigation. So far as is known to the writer, the field of enquiry is still entirely unexplored in respect to the mycorrhiza of the plants belonging to these families.

EXPLANATION OF PLATES VI AND VII

PLATE VI

Calluna vulgaris

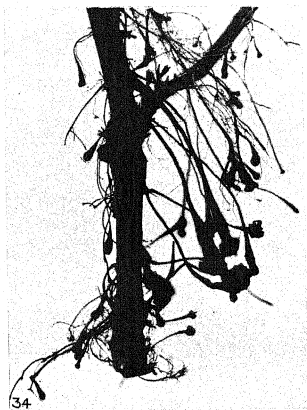
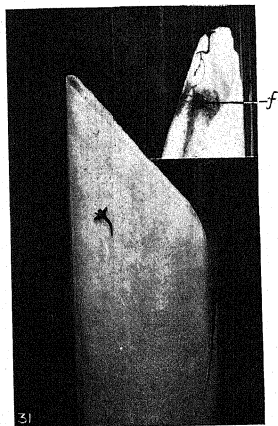
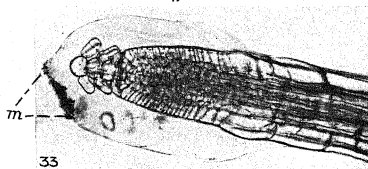
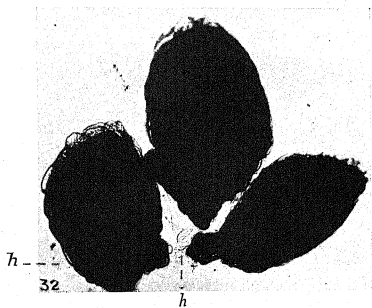
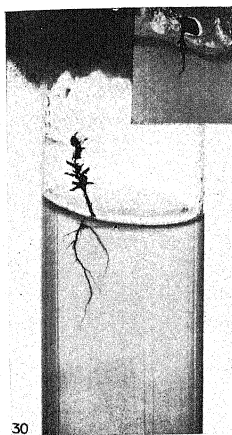
- Fig. 30. Seedling raised from sterilised seed; growing in nutrient agar inoculated at planting from pure culture of the endophyte, 27 days from planting. *Inset*, seedling of same age uninfected.
- Fig. 31. Seedling raised from sterilised seed; in aseptic culture on filter paper, 27 days after removal from seed-dish. *Inset*, seedling of same age inoculated at planting. *f*, mycelium of endophyte.
- Fig. 32. Seeds removed from capsule before dehiscence, showing fungal infection of the seed-coats. *h*, hyphae.
- Fig. 33. Tip of young root, showing mucilaginous sheath. *m*, mucilage.
- Fig. 34. Base of main stem, showing formations of tubercle roots.

PLATE VII

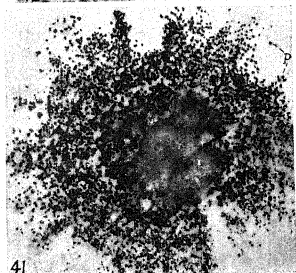
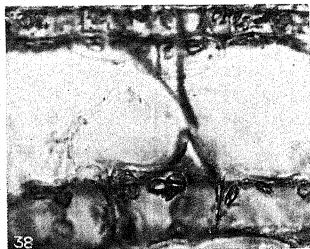
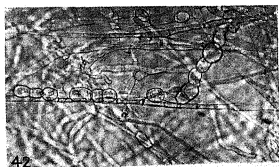
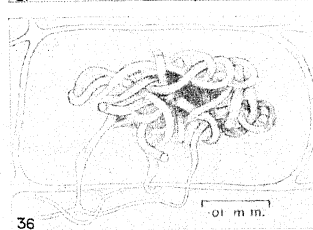
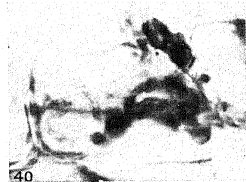
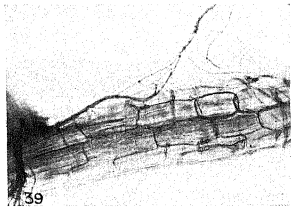
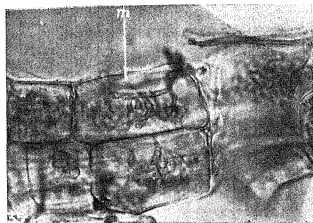
Calluna vulgaris

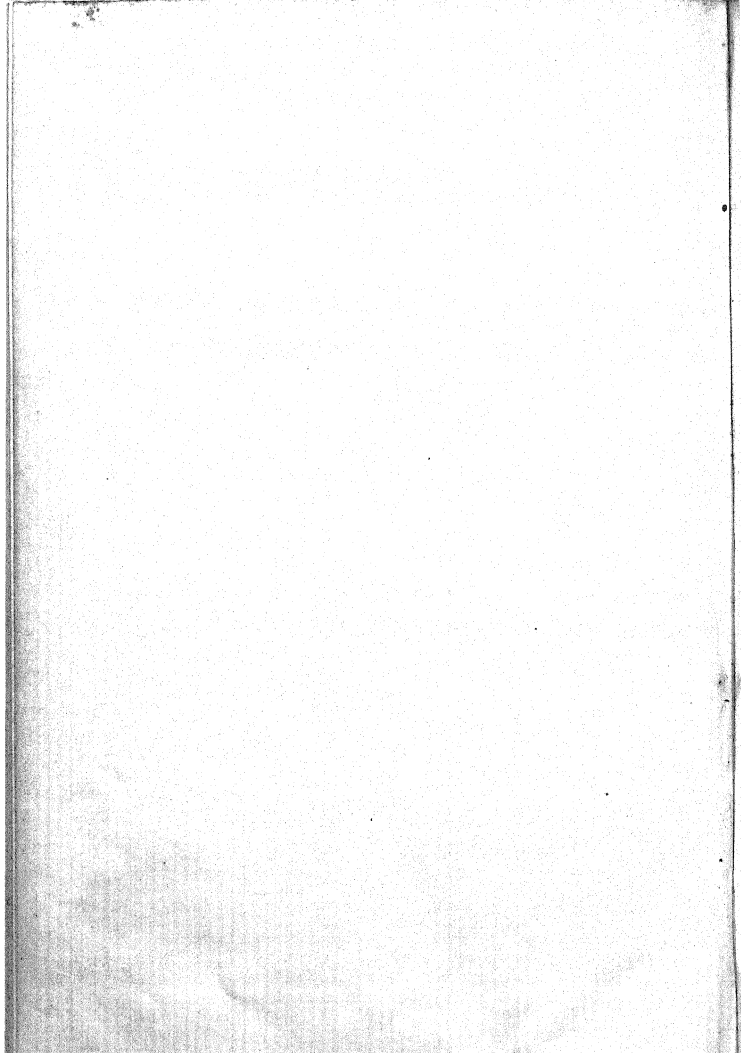
- Fig. 35. Mycorrhiza cells of root: mycelium in active condition; *m*, mycelial complex. $\times 1620$.
- Fig. 36. Mycorrhiza cell in very early stage of digestion; "clumping" of mycelium. $\times 1350$. (From Rayner, *The British Journal of Experimental Biology*, 1925.)
- Fig. 37. Mycorrhiza cells in advanced stage of digestion. $\times 900$. (From Rayner, *The British Journal of Experimental Biology*, 1925.)
- Fig. 38. Mycelium in stem tissues. Fine hyphae in cells of pith extending into wood. $\times 1600$. (From Rayner, *The British Journal of Experimental Biology*, 1925.)
- Fig. 39. Young root of cutting (cf. Fig. 29) rooted in sterilised sand, showing typical fungus infection. $\times 325$. (From Rayner, *The British Journal of Experimental Biology*, 1925.)
- Fig. 40. Cell from young root of cutting rooted in sterilised sand, showing intracellular mycelium and formation of "suppressed mycorrhiza." (From Rayner, *The British Journal of Experimental Biology*, 1925.)
- Fig. 41. The endophyte of *Calluna*, *Phoma radicis callunae*: sporing culture in dextrose agar, three months old; *p*, pycnidia.
- Fig. 42. *P. r. callunae*: mycelium from old culture (36 days) in dextrose agar.

(To be continued)









COMMENTS ON THE THEORY OF THE SOLID CARPEL AND CARPEL POLYMORPHISM

By JOHN PARKIN

IN a measure botanical science at the present day may be said to be at the crossways. Facts supposedly well established and opinions long held are being questioned. Instances may be given by way of illustration. Specific differences are no longer in some quarters being looked upon as adaptive, but as chance occurrences—mere embellishments—which provided they are not detrimental are retained though of no actual use to the plant. The idea of evolution by gradual change is being set aside for that by sudden jumps—mutation. Structures regarded as evolved to resist dryness are being doubted as functioning so, and physiological drought is said to be a fiction. The erstwhile view that the xylem and phloem perform distinct conducting rôles is being attacked by two opposing schools. The one claims that the wood vessels not only convey the mineral salts, but also manufactured food materials such as sugars; while the other brings forward experimental evidence in favour of the novel idea that the sieve tubes conduct not only the so-called elaborated sap, but also the greater part of the inorganic nitrogen as well. Quite recently the usually accepted interpretation of the syncarpous gynoecium has been seriously challenged by the theory of the solid carpel¹. A critical review of this theory is herein attempted.

It is well that from time to time there should be a stocktaking—a fresh appraisalment—of our botanical generalisations. A finer technique and the progress of science generally offer a sufficient inducement for the arraignment anew of current text-book statements before the bar of observation and experiment. Some supposed facts may thereby be more firmly established, others modified and others again possibly rejected. At the present time, however, a cynical onlooker might be tempted to suggest that botanists are unintentionally striving to fix more securely the foundations of their science by the Euclidean *reductio ad absurdum* method!

¹ Saunders, Edith R., *Ann. Bot.* 37, p. 451, 1923; *ibid.* 39, p. 123, 1925, and *New Phyt.* 24, p. 206, 1925. The paper in the *Annals of Botany* published in 1923 is referred to under the letter A, and the one published in 1925 under B. The paper on the Rosaceae in the *New Phytologist* is not referred to in detail.

Whether the theory of the solid carpel merits in the least such a gibe, it is naturally too early to say. But at any rate it appears to the writer that this theory, worked out and applied with so much ability, ingenuity and assurance by Miss E. R. Saunders, should neither on the one hand be lightly passed over, nor on the other hand accepted and incorporated into our science without much scrutiny; for it not only revolutionises our ideas respecting the syncarpous gynoecium, but also imposes on the student a greater difficulty than there is at present in grasping the structure of such gynoecia. Before burdening him so, let us have some unanimity of opinion as to the correctness of Miss Saunders's contentions.

The theory was primarily applied to the elucidation of the difficult Cruciferous gynoecium. At first, I may confess, I was distinctly drawn towards her view that this gynoecium consists, instead of two carpels, of four, viz. two valvular and two solid, forming together the placentas and false septum. When later, however, she proceeds to find these solid (or semi-solid) carpels in a number of families far apart from the Rhoeadales one becomes sceptical. It is a wrench with the past to have to write, for example, G_2 instead of G_1 for the Leguminosae, G_{10} instead of G_5 for the Primulaceae, G_6 instead of G_3 for many Monocotyledons, and, in the case of individual genera, G_{16} instead of G_2 for *Brassica* and G_{20} instead of G_2 for *Eschscholtzia*. Such sweeping changes naturally call for caution and for a close examination of the facts and reasonings upon which they rest.

Miss Saunders tells us she was led to the theory of carpel polymorphism through the study of abnormal fruits of the stock (*Matthiola incana*)—a plant, as we know, which she has made peculiarly her own from the Mendelian standpoint. She regards these fruits as reversionary, pointing to the ancestral Crucifer as having double the number of carpels normally present in existing Cruciferae. It may be so. Had her theory, however, depended mainly upon teratological structures, one would have been inclined at this point to urge the danger of this kind of evidence. There is the temptation to use for our argument such abnormalities as suit our purpose and to leave on one side those that do not; for it is obvious that many sports cannot be regarded as atavistic. Until we can distinguish between reversionary and what, for the want of a better word, one might term pathological, sports, it would appear safer to leave out teratology or to call in its aid merely as subsidiary.

However, it is plain that Miss Saunders relies mainly on vascular

evidence for her theory. To my mind what is lacking is clear comparative evidence, showing the evolution of a functional (in the sense of bearing stigma and ovules) solid carpel. That there may be vestiges of carpels, at a former period functional, in certain flowers may be admitted. The only clear example brought forward is that of *Triglochin* (*Helobieae*). In *T. maritimum* there are six fertile carpels, and in *T. palustre* three fertile and three sterile alternating with them. These latter, as the author states, "though still enclosing a cavity, are obviously much reduced and on the way to becoming solid" (A, p. 476). This evidently is a genus showing incipient syncarpy. Its allies are apocarpous. *Triglochin* suggests that syncarpy with axile placentation can arise directly from apocarpy¹. This is fairly evident when one thinks of a circle of follicles (valvular carpels) cohering together. Such primitive capsules would also naturally be septicidal. As shown by the above quotation, the author hints at the way a solid carpel may be formed out of a locular one composing part of a syncarpous gynoecium with axile placentation. Full compression will result in the obliteration of the loculus and it is conceivable that the carpel might persist as a vestigial vascular cord. It is even possible that such a remnant might be retained to help in some way in dehiscence as is suggested. But it is extremely difficult to see how such a compressed (solid) carpel could retain its fertility, i.e. continue bearing ovules, though it might possibly keep its style and even stigma. Apparently carpels bearing ovules and considered solid are supposed to have arisen in this way.

But probably Miss Saunders may, in the main, surmount the foregoing difficulty by imagining the derivation of solid carpels as a rule from gynoecia with parietal placentation. The solid carpel (now somewhat of a misnomer) is considered to arise through the disappearance of the valvular (laminar) part of the carpel leaving only the midrib. As the two halves of the carpellary leaf shrink in width, the ovules borne on their margins will gradually be brought nearer to the midrib and finally, when the "solid" state has been reached, they will appear to be borne on the midrib. A fertile solid carpel formed in this way is conceivable. The question is, does it exist? Miss Saunders relying chiefly on vascular evidence considers it of frequent occurrence throughout the Angiosperms.

¹ It is probable that syncarpy with parietal placentation has at times arisen from apocarpy directly without the intermediate stage of syncarpy with axile placentation. The Papaveraceae and Resedaceae for example suggest this. Many axile placentations would appear to be secondary and to be derived from parietal ones by the ingrowth of the placentas.

As already pointed out the fertile solid carpel was first postulated for the Cruciferae. The immediate Cruciferous ancestor is held to have had a 4-valvular carpellary ovary with parietal placentation¹. The actual Cruciferous gynoeceium resulted from one pair of opposite carpels remaining valvular but losing their ovules, and the other pair becoming solid by reduction, but remaining fertile, with the ovules borne close to the midribs. From each of these midribs an ingrowth took place, the two fusing to form the false septum (replum). This is the structure which presents difficulty in any interpretation of the Cruciferous gynoeceium. The old or current view is that the replum has been formed by ingrowths from the two placentas fusing—a view in my estimation at least as probable as the foregoing and not requiring any new theory.

Multicarpellary ovaries in the Cruciferae. In her second paper the Cruciferae are defined as having four, eight or many carpels. Let us glance at the examples given of genera and species with more than four (i.e. two according to the current view). For the purpose of our argument a paragraph of the summary dealing with the Cruciferae is here reproduced (A, p. 480):

Ovaries of more than four carpels generally show a number of furrows, ridges, ribs, wings or prominent parallel veins corresponding with the number of the solid or of the valve carpels present. Thus, e.g. in the genus *Isatis* the contour of the fruit in one species indicates the presence of six valves, in another of ten. From the character of the veining the number of carpels in species of *Brassica* and *Sinapis* appears to be sixteen; from the number of wings, sixteen, in *Guiraoa arvensis* Coss.; from the ribbing, between forty and fifty in species of *Rapistrum*. It is necessary, however, to distinguish between formations associated with *parallel* veining and other outgrowths produced as the result of the presence of strong *lateral* veins, such as the horns in *Tetracme*, which are not significant of the carpel number.

¹ It is difficult to grasp completely the author's views as to the ancestral forms of the Cruciferous gynoeceium. To quote (A, p. 481): "The widespread occurrence of reversionary fruit types indicates that the typical 4-carpelled siliqua and silicula have been derived by reduction and consolidation from an earlier ground-plan, in which $G=4$ (hollow) + 4 (solid); which in turn arose by simple reduction from one composed of a much larger number of both kinds of carpels, this construction being yet again the result of consolidation from an all valve type with G numerous." It is manifest from the paragraph which follows this that the typical G_4 condition is regarded as derived from the G_4+4 state by the total suppression of the second (upper) whorl of carpels. It is not, however, clear to me whether all these supposed ancestral gynoecea are to be looked upon as syncarpous, or whether the earliest were apocarpous. If syncarpous then how has arisen the double-whorled condition? Diagrams upon which to fix one's thoughts would have been useful here.

It is clear from the above that the evidence for the increased number of carpels is derived from the extra number of *longitudinal* parallel vascular strands. Each of these, one imagines, is supposed to represent the remnant (midrib) of a once functional valve carpel. When, however, a vascular bundle is strengthened in the *lateral* direction, it is not looked upon as having any carpellary significance. Logically why need it have this significance when it runs in the longitudinal direction; especially when it involves the difficult conception of the gynoeceium of *Rapistrum* consisting of forty to fifty compressed carpels? This supposition of additional carpels up to as many as fifty in certain Cruciferae conflicts strongly with the systematics of the family. The other organs of the flower are with very few exceptions fixed numerically throughout this sharply defined group. Taking floral morphology as a whole, the stamens vary more in number than the carpels; but if Miss Saunders's views be correct the Cruciferous flower is an exception. One would like to know how this supposed multicarpellary condition has come about. From the instances given it can hardly be argued that a primitive feature has been retained, though lost in closely associated forms. One has, it would seem, to fall back on the saltatory idea, viz. a sudden increase in the number of solid carpels. I fancy even a thoroughgoing mutationist might pause here!

The Commissural Stigma. The occurrence of the stigma over the suture instead of above the midrib has occasioned some difficulty in the past in the elucidation of the gynoeceium of the Cruciferae and allied families. Miss Saunders by means of her theory of the solid carpel apparently removes this difficulty; for the stigma is now represented as invariably placed over a carpellary midrib. This is one of the most appealing points of her theory. Yet one or two criticisms may be ventured here, which if acceptable may render unnecessary the hypothesis of carpel polymorphism to explain the commissural stigma.

In the Cruciferae the stigmatic papillae are by no means invariably centred over the sutures. This is admitted in the first paper, e.g. for the Stock, where the stigmas are said to be over the midribs of the valves. This somewhat awkward position is dismissed by the following felicitous passages:

Hence, we need no longer subscribe to the time-honoured morphological fiction of the commissural stigma. In place of it, we have clearly shown to exist a morphological dimorphism sufficiently elastic to permit of a considerable degree of physiological interplay without change of ground-plan (A, p. 466).

As polymorphism arose, the stigma-bearing and the ovule-bearing functions often came to be performed by one kind of carpel only. But though the distribution of stigmas and placentae among the different carpel members usually became constant for the species and often for the whole genus, in neither case did it appear to be the result of any fixed interrelationship between one carpel type and another (B, p. 130).

This "physiological interplay" and lack of "any fixed interrelationship between one carpel type and another" is somewhat subversive of comparative morphology. In such a circumscribed and natural group as the Cruciferae, if there be this carpel polymorphism, one would have expected the two types of carpel to have retained throughout their distinctive characters—the valves continuing sterile and the solid carpels fertile (stigma- and ovule-bearing).

It is interesting with regard to the commissural stigma to note that Prantl in his classification of the Cruciferae in the *Pflanzenfamilien* (3, 2, p. 154) uses the position assumed by the stigma as a major character for dividing up the family. On scrutinising his arrangement on these lines, the idea suggests itself that a spreading capitate stigma may be primitive for the Cruciferae and a commissural one derived through a restriction of the stigmatic papillae to the areas over the sutures. If the restriction occur in the opposite direction, then the papillae will be centred over the midribs, thus accounting for those supposed exceptional genera, *Matthiola*, *Moricandia* and *Lonicophora* mentioned in Miss Saunders's first paper. A point in favour of this view is perhaps apparent in the statement that in "the young siliqua [of the stock] before the sutural knobs enlarge they [the stigmatic papillae] appear as an almost complete ring" (A, p. 462).

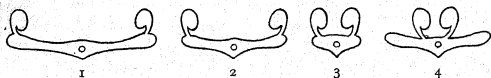
Possibly a close comparative study of the Papaveraceae might reveal that in this family the commissural stigma is also a derived structure. In *Papaver*, probably an advanced genus, the characteristic sessile rayed commissural stigma may have come from a stalked rayed one, and this, in its turn, through lobing from a capitate one. If so there would be no need of introducing carpel polymorphism to explain the stigmatic position in this family.

Dehiscence. A strong point is made by Miss Saunders of the lines of dehiscence in the fruit of the Cruciferae, as evidence for the 4-carpellary structure of the gynoecium. This is considered as a rule to take place down the lines of juncture between the valves and the supposed solid carpels. It will generally be admitted that the primitive mode of dehiscence of the carpel is along the line of

the ventral suture. It is so in the follicle. When a whorl of free carpels cohere to form a syncarpous gynoecium, primitively the dehiscence will follow the same lines, resulting in a septicidal capsule. As syncarpy progresses, the demarcation between the individual carpels will become less and less clearly defined, and the dehiscence will tend to take on a variety of forms and be no longer restricted to the ventral suture. It will thus cease to be reliable as an indication of carpel boundaries. Such is the way the writer is inclined to regard the matter from the evolutionary side. It is clear in some cases that the line of dehiscence cannot be taken as that of the carpel boundary, e.g. in the pyxidium, where the dehiscence is transverse.

Miss Saunders herself, in being true to her interpretation of gynoecial venation, has to admit that in the silicular Cruciferae the lines of dehiscence do not follow the lines delimiting her two kinds of carpels, which in the silicula are considered to be valvular and semi-solid (instead of solid as in the siliqua). This admission appears to me to weaken the 4-carpellary interpretation of the Cruciferous gynoecium.

Semi-solid Carpel. If the siliqua has preceded the silicula in the course of evolution, which seems likely, then in the Cruciferae the semi-solid carpel must have originated from the solid kind. Miss Saunders even concedes this. To quote: "Nor need we be surprised at this order of development (valve—solid—semi-solid), since we are familiar with many instances of mutations in which the larger change is made first, the intermediate stage not appearing until later" (B, p. 130). But apparently she also regards the semi-solid as at times an intermediate stage between the valve and the solid form. Yet whichever way the semi-solid carpel is evolved, it has the same structure according to her definition. Let me explain the position by means of simple diagrams:



It might have been imagined that the semi-solid carpel, derived directly from the valvular (Fig. 1) through shrinkage, would have retained its ovules on the margins as depicted in Fig. 2, and not have shifted them to the midrib (Fig. 4). With the complete disappearance of the valvular sides, the ovules would then perforce appear as if borne on the midrib (Fig. 3). When, on the other hand,

the semi-solid is derived from the solid form, the ovules would still retain their midrib position (Fig. 4), as the lateral expansions are presumably new outgrowths. This identity of structure between what we might call semi-solid and pseudo-semi-solid carpels casts doubt upon the existence of these kinds of carpels and weakens the case for that of the solid carpel itself.

So far space has chiefly been occupied in criticising the theory of carpel polymorphism as applied to the Cruciferae. If the theory can be shown to be inapplicable to this family, then *a fortiori* its application to other types of gynoecia (save perhaps those of the Papaveraceae) becomes still less likely. However, some points raising difficulties connected with other families reckoned to contain solid or semi-solid carpels deserve attention. Before going on to these let me add a remark or two on the Papaveraceae.

Papaveraceae. The commissural stigma, so characteristic of the higher genera of this family, has already received comment. Much capital is made of *Eschscholtzia* and its supposed allies, *Dendromecon* and *Hunnemannia*¹. They are considered to have a gynoeceium composed of ten solid and ten other carpels, instead of only two according to the current view; only a pair out of these twenty carpels bear ovules. As the fruit when ripe splits solely into two parts, these genera have given rise to the conception of the *compound valve*.

The strongest piece of evidence in favour of the multicarpellary nature of the gynoeceium of *Eschscholtzia* lies in the frequent presence of more than two stigmas—twelve may even occur. The solid carpel theory offers a ready explanation of these additional stigmas; but perhaps a thorough comparative study of the Papaveraceae as a whole might explain these extra stigmas without having recourse to this intricate theory.

Resedaceae. The theory as applied to the Resedaceae brings out some rather astonishing and perplexing points as regards the gynoeceium of the genus *Reseda* itself. The page or more devoted to this family is difficult reading (A, p. 472). We learn that in certain species, e.g. *R. luteola*, the carpels are all of the valve type and that the double placenta bifurcates above. There will be general agreement here. The gynoeceium in fact is apocarpous in the upper part, and the placentation being parietal the ovary is open at the top—a striking feature in this family and one almost unique. Miss Saunders now proceeds as follows:

¹ In the latest systematic arrangement of this family (Hutchinson, *Kew Bull.* p. 163, 1925) *Eschscholtzia* is placed apart in a tribe by itself.

In other species in which the double placenta remains whole, as e.g. in *R. lutea* L., *R. alba* L., *R. phyteuma* L., the sutures present a double contour line—they are in fact not merely placentae but whole (solid) carpels and hence remain intact (A, p. 473).

The ordinary interpretation is that the gynoeceum in *R. luteola* and its allies is in a less advanced stage than in *R. lutea* and its associates. The one represents incipient syncarpy on parietal lines, and the other a rather more evolved stage in which the fusion between the adjacent carpellary edges has proceeded further. In two other genera, *Caylusea* and *Astrocarpus*, the carpels are actually free—a still earlier stage presumably. Here, one supposes, there is no question of solid carpels being absent.

Further on we read:

It is now also clear why even the young syncarpous gynoeceum in *Reseda* is open at the top. The valve carpels are joined to the intervening placentiferous solid carpels, but the latter do not extend to the same height as the valves; consequently where the solid carpels cease, the valves are disjoined and the ovarian cavity is not closed in (A, p. 473).

This explanation, however, introduces a difficulty. Why is the gynoeceum of *R. luteola* with only valve carpels open at the top? This is recognised and got over by the supposition that the open type of ovary became fixed before the disappearance of the solid carpels. We are to believe, then, that a form like *R. luteola* had once solid carpels which have been lost. We are inclined to ask here whether the ancestors of the *luteola* group of species are supposed to have had three fertile or three sterile valve carpels as well as three fertile solid members. If sterile then presumably on the disappearance of the solid carpels, the valves took over the ovules!¹

Incidentally the Resedaceae lend support to the view suggested in this paper that the commissural stigma is a derived rather than a primitive feature. *Reseda odorata*, probably an advanced species, is said to possess such a stigma, whereas in *R. luteola*, primitive as regards its gynoeceum, the stigmatic papillae are considered to be centred over the midribs of the valves. Perhaps primitively the stigmatic surfaces were diffuse and to some extent decurrent; and later became restricted to definite areas.

¹ In her second paper the gynoeceum of *R. luteola* and its associates is no longer regarded as consisting of valve carpels, but rather of three solid and three semi-solid ones. When penning the above remarks on the Resedaceae I had overlooked this change of view, and the criticism may now cease to apply; but it is left as originally written as I am still somewhat uncertain as to the delimitations of the supposed six carpels in *Reseda*.

Leguminosae. Though Miss Saunders's first paper startled us, her second has added still more to our astonishment, for this large family has been pronounced to have an ovary composed as a rule of two instead of the generally accepted one carpel. We hoped the Leguminous pod would have been above suspicion, but it is not so. In fact a true valvular carpel is not considered to be present at all—only those of the solid and semi-solid types.

Haematoxylon is first brought forward in proof of the 2-carpellary interpretation. In this genus the pod dehisces down the middle of each flat side, instead of along the two sutures—the usual way. This exceptional mode of dehiscence is explained by supposing that the gynoeceium is made up of two semi-solid carpels and that the splitting of the fruit follows the lines of juncture. Only one of these carpels is deemed fertile, bearing ovules on its midrib. The gynoeceium of the majority of the *Leguminosae* is supposed to consist of a fertile semi-solid and a sterile solid carpel. It is strange that *Haematoxylon*, which from other considerations can hardly be reckoned a primitive genus, should differ in its carpellary composition from the remainder of the family!

In the *Leguminosae* as in the *Cruciferae* examples are given of genera and species with multicarpellary ovaries on the grounds of the occurrence of a number of marked longitudinal vascular cords in the fruit wall. Each of these strands, often corresponding outwardly to a rib, is considered to represent a carpel reduced to the solid state. *Arachis hypogaea* (the monkey nut) is held to have 10–12 of such carpels and *Scorpiurus* 10–14 (B, p. 144). It is indeed odd that such advanced genera (*Papilionatae*) should have retained the multicarpellary ancestral condition, while their associates conform to the normal Leguminous plan! Miss Saunders's supposed multicarpellary genera introduce, therefore, a phylogenetic difficulty.

Liliaceae. The 6-carpellary view of the monocotyledonous gynoeceium is strongly upheld for the petaloid families; and one imagines that the *Liliaceae* are somewhat crucial for the maintenance of this standpoint. They are generally considered less evolved florally than the *Amarylhidaceae* and the *Iridaceae*, and certainly than the *Orchidaceae*. Towards the end of the discussion of the *Liliaceae* the following paragraph occurs:

In all the genera hitherto dealt with the trend has been in the direction of more or less consolidation of all the carpels with retention of both whorls. In certain genera of the section *Melanthioideae* we appear to meet with the converse condition—no con-

solidation of the outer whorl, which retains the valve form, but reduction of the total number by complete disappearance of the inner whorl, as e.g. in *Melanthium virginicum* L., where $G = 3$ typical fertile stigma-bearing valve carpels (B, p. 166).

Now this is an interesting and, from my point of view, a weakening admission. The Melanthioideae are probably a primitive group of the Liliaceae. If solid carpels be really present in this family, we might have expected signs of their evolution here, but apparently there are none. On the current view of the 3-carpellary nature of the Liliaceous gynoecium, septicial dehiscence of the capsule, found in this tribe in distinction to the loculicidal type occurring elsewhere in the family, is just what might have been expected on the view that the former kind of splitting preceded the latter in evolution.

Such criticisms as have been offered are largely of a comparative or phylogenetic nature, and little has been said in the way of explaining the presence of those vascular strands upon which the theory of the solid carpel mainly rests. The writer has to confess that he has not to any extent examined for himself the material used by Miss Saunders, nor has he seen any of her preparations. Respecting vascular cords he would like to make this observation. May not increased needs either of water or food or of both to certain parts tend to strengthen and even straighten the vascular tissue supplying such regions? Such a view is supported by the fact mentioned by Miss Saunders that the horns on the fruit of the Cruciferous genus, *Tetrameles*, are supplied with strong *lateral* veins. These vascular strands she does not regard as carpellary significance; yet where they run *longitudinally*, supplying ribs, wings etc., she does so.

This attempt at a criticism of the theory of the solid carpel or carpel polymorphism is put forward in no carping spirit, but in the interests of floral morphology and evolution. As already expressed, the theory appears so revolutionary of our ideas of gynoecial composition that it would be unwise to accept it at its face value without realising the commitments to which it exposes us. At the same time it is quite possible that the writer has in some respects misunderstood the theory and so misdirected criticism. If so, he can only plead in extenuation that he has found the subject by no means easy to grasp.

A COMPARISON OF THE JUVENILE AND ADULT LEAVES OF *EUCALYPTUS GLOBULUS*

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(With 5 figures in the text)

IT is well known there is an extraordinary difference between the young and mature foliage of *Eucalyptus globulus*; a difference which includes variation in texture, size and insertion, but particularly a difference in position. It is this difference in position which has given rise to a considerable amount of speculation as to the reason why the adult leaves assume a vertical position. It is generally accepted that the horizontal position, which is that of the leaves in the young plant, is the original one.

Why, it has been asked, in accordance with the teleological standpoint, have the leaves adopted the vertical position? Is it in order to protect themselves against too great intensity of light, which may be harmful to the tissues, or has the determining factor been an urgent need for diminishing the loss of water? In other words, is it a case of protection against insolation or against transpiration?

It is impossible to show positively that either of these supposed advantages, working through natural selection, has been the *cause* of the development of the vertical leaves, but if it can be shown that either of them does not in fact exist, that is actually evidence against the corresponding theory.

There is consequently a considerable interest in a comparison, both of structure and function, between these two types of leaves. It was this interest which led to the following investigation, in order to ascertain whether the leaves exhibited structural features which might account for any difference in the rate of transpiration.

Maiden⁽²⁾ points out that Henslow⁽¹⁾ and Schneider⁽³⁾ differ in their descriptions of the anatomy of *Eucalyptus* leaves, and he considers this to be evidence of variation, pointing out at the same time, however, that the leaves of *Eucalyptus* offer much room for research.

The material used for the investigation was obtained from two plants growing in a greenhouse at Fallowfield belonging to the

Manchester University. Part of one plant only had developed the adult type of foliage, the remainder of the leaves on the plant consisting either of the very young forms of the juvenile type, or of transitional forms between the juvenile and adult. The other plant bore principally leaves of the juvenile type, only a few transitional types being present in addition.

The juvenile leaves, which are produced by the plant in its earlier years, are borne so that their upper surfaces are at right angles to the rays of the sun, i.e. in the normal position of leaves—horizontal (see Fig. 1 in text). They are without petioles, cordate at the base,

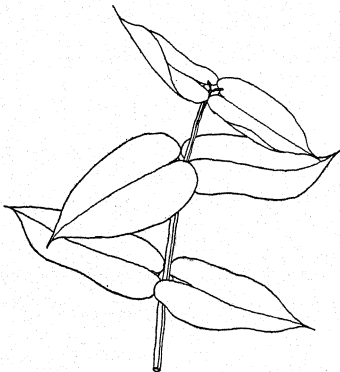


Fig. 1. Juvenile Leaves. ($\times \frac{1}{2}$.)

with a short sharp point at the apex, and are about twice as long as broad. They are produced by branches which are square and winged, and are arranged in pairs, at right angles to each other. Both stem and leaves possess a thick coat of wax, which is much thicker on the under than on the upper surface of the leaf. The very young forms of the juvenile leaves are larger, thinner, and less waxy, and droop more rapidly than the older juvenile leaves when detached from the tree.

The adult leaves are produced later in the life of the tree. They are consequently less likely to be shaded than the juvenile and are of a totally different type. They are borne spirally on a rounded

stem, are sickle-shaped, very thick and leathery. The petioles have twisted through an angle of 90° , and thus the leaves have no longer a horizontal position, but are vertical, presenting their edges to the rays of the sun. They are devoid of that heavy coating of wax which is so characteristic of the juvenile leaves (see Fig. 2 in the text). Some of the older cotyledons which are small and



Fig. 2. Adult Leaves. ($\times \frac{1}{2}$)

bilobed have just a trace of wax on the under surface, but in their youngest stages they have none at all on either surface.

The material investigated showed, between these two very distinct types of leaves, others of a transitional form, showing transitions in shape and arrangement, and also variations in the amount of wax present.

The waxy substance covering the leaves consists of a continuous layer of material raised into numerous little heaps. Transverse sections of the leaves show these heaps to consist of a number of rods which are sometimes straight and sometimes curved in different directions. The surfaces of these rods which stain red with Sudan III are in some cases smooth, but in others there are little collections of minute and distinct granules attached to them. The rods themselves are readily soluble in xylol, but only a portion of the basal layer, in which also could be distinguished small granules, dissolves (Fig. 3 in text). A description of these will be found in Briosi(5).

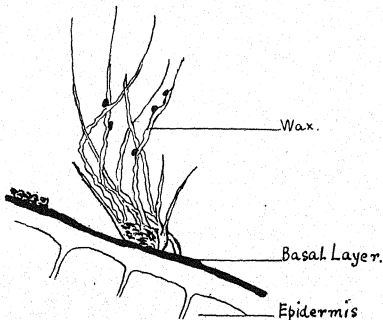


Fig. 3. Wax Rods. ($\times 900$.)

Exudations from the oil glands were present on all the leaves. Sometimes they had the appearance of resinous-looking club-shaped bodies standing up from the surface of the leaf. In other cases they had flattened down a little, whilst sometimes they had formed a thin film over part of the surface of the leaf. All these often remained present in leaves which had been standing in xylol all night. In addition to these slimy resinous-looking exudations, white irregular masses of small granules were seen on all the leaves, after the wax had been removed with xylol. Sometimes these masses were seen standing erect, large or small, directly over an oil gland. In other cases they were scattered about the surface of the leaf. Occasionally they took the form of little upgrowths of granules, and sometimes they had the appearance of long threads. It appeared that they were all probably due to the oily secretion from the very

numerous oil glands present in all the leaves, and Naudin's statement concerning the exudation of the resinous secretion supports this (Maiden⁽³⁾).

The adult leaves are considerably thicker than the juvenile ones, measuring respectively .3723 mm. and .2145 mm. near the edge and .4196 mm. and .2343 mm. near the midrib. Whilst on the one hand the juvenile leaf is covered with a thick coating of wax over a thin cuticle, the adult leaf possesses a very much folded cuticle, approximately three times as thick as that of the juvenile, and is without wax. The folds of cuticle are regular in so far as there is one papilla to each cell, but irregular in so far as the papillae are not by any means always over the centres of the cells. The cellulose portion of the cell wall follows the outline of the cuticle, and therefore has a papilla immediately under the cuticular papilla. Transverse sections of the leaves show that the cuticle in the adult comes down between the epidermal cells in well-marked points, whilst in the juvenile leaves this is not the case. The epidermis of the juvenile leaf is much more regular in outline and has only very small papillae, scarcely evident in some of the cells, when seen in transverse section.

I found very little difference in the thickness of the upper and lower epidermis, although of course the epidermis of the adult leaf is considerably thicker than that of the juvenile as the following measurements show:

	Lower epidermis	Upper epidermis
Adult:	.0236 mm.	.0251 mm.
Juvenile:	.0161 "	.0176 " (Schneider (3); Henslow (1))

Surface views of the epidermis of both types show that they are polygonal in shape and variable in size. Furthermore each cell of the adult shows, sometimes near the centre, sometimes near the walls, a definite circle of transparent material with a few granules in the centre, and around this a darker irregular mass. This appearance of a circle in each cell is very noticeable, and is in fact very like the appearance of the lens-shaped thickenings of the cuticle of *Garrya elliptica* figured by Wager⁽⁴⁾¹.

¹ The similarity of the lens cells in the adult to those figured by Wager (4) (though in the case of *Garrya elliptica* they appear to be quite independent of the cells, whilst in *Eucalyptus globulus* there is one to each cell) led me to see whether the lens-like structure in *Eucalyptus globulus* would produce a clear image. I prepared sections of the epidermis as described by Wager and discovered that I not only got a very clear image of any small object held in position correctly, but, on moving the object slightly, obtained two and sometimes three images in a cell. In the case of the juvenile leaves, the lens-

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The stomata vary both in size and distribution. The horizontal leaves have stomata only on the under surface, with the exception of a few scattered ones on each side of the midrib. The vertical leaves have them on both surfaces.

Number of stomata per sq. mm.: Adult (vertical)	85.995
Juvenile (horizontal)	83.785

As the above figures show there is not a great difference in the numbers of the stomata in the two types of leaves. The same cannot, however, be said of the size, as the average of the readings in this case showed the stomata of the adult leaves to be nearly twice the size of those of the horizontal.

	Length	Breadth
Adult: Upper surface	.053 mm.	.043 mm.
Lower "	.054 "	.041 "
Juvenile: Lower "	.029 "	.0236 "

Many of the stomata in the vertical leaves display some very interesting features. The cells of the palisade tissue surrounding the air space are considerably modified. One of these cells grows out into the air space, until it more or less assumes its shape. Its walls come quite close to those of the guard cells, and in fact, in many cases, it almost closes the air space. Its upper wall is very much thickened and cutinised and the whole of the air space is also cutinised. These special cells contain protoplasm and a vacuole, but no chlorophyll (Fig. 4 in text).

The guard cells of such stomata also are modified, they have very little chlorophyll and are very much flattened. I have not found any such cells in the horizontal leaves, nor does Briosi(5) record their occurrence.

In both types of leaves the cuticle projects above the guard cells, is drawn out to a fine point—more produced in the vertical leaf—and thus forms a chamber over the pore. In the vertical leaf the cuticle extends in a thin layer over the outer surface of the cells

like thickenings of the cuticle are much more difficult to find, but I have seen them on both surfaces of the leaf, although they were smaller on the lower surfaces. In both cases images were obtained.

These facts are interesting when one considers the very different positions of the two types of leaves, with reference to the incident rays of light.

The fact that the lens-shaped thickenings of the cuticle are so much more pronounced in the vertical leaves of *Eucalyptus globulus* than in the horizontal, appears to me to support the contention of Wager (4) that such lens-like thickenings are not necessarily organs for setting up a stimulus, which results in the leaf obtaining a better position with regard to the incident rays of light (Haberlandt (7); Wager (4)).

bordering the air space, but in the horizontal leaf it only extends in a very thin layer indeed, to the point where the epidermal cells adjacent to the guard cells meet the mesophyll.

The mesophyll of the two types of leaves shows considerable differences in thickness and structure. That of the vertical leaf is much thicker and shows a distinct palisade parenchyma on both surfaces. There is, however, a difference between the palisade of the morphologically upper surface and that of the morphologically lower surface. Below the upper surface there are two, and sometimes three rows of rather large clearly defined palisade cells, the outermost row with inconspicuous air spaces, whereas next the lower surface there is one layer of closely packed smaller palisade cells

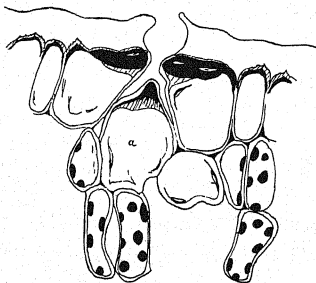


Fig. 4. T.S. Vertical Leaf showing modified palisade cell (a). ($\times 600$.)

followed by a layer of less clearly defined and less closely packed palisade cells.

Upper palisade	·0461 mm.
Lower „	·0277 mm.
Tissue between	·2291 mm.

Between these the cells are more loosely packed, but still somewhat elongated, leaving rather long narrow air spaces between. Thus there is not the differentiation into the palisade and spongy parenchyma, nor the same amount of air space as in a typical dorsiventral leaf.

In the case of the horizontal leaf a differentiation into palisade and spongy parenchyma can be observed. There is one definite layer

of closely packed palisade cells on the upper surface, and a layer of rather elongated, but somewhat irregular cells on the lower surface, which Briosi(5) calls "pseudopalisade." Between these the cells are more loosely packed and less elongated, leaving more rounded air spaces than in the vertical leaf.

Upper palisade	·0326 mm.
Lower "	·0191 mm.
Tissue between	·0727 mm.

Near the lateral vascular bundles, however, the mesophyll of the two types is less distinct, that of the horizontal leaf approaching that of the vertical (Fig. 5 in text).

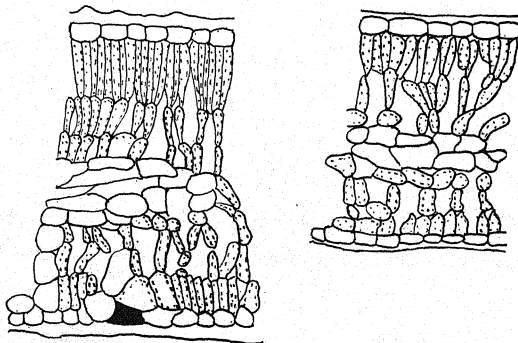


Fig. 5.

T. S. Vertical Leaf near vascular bundle. ($\times 200$.)

T. S. Horizontal Leaf near vascular bundle. ($\times 200$.)

In proportion to the amount of assimilatory tissue present, the horizontal leaf has a larger area of air space than the vertical leaf, as shown by the following figures:

	Area of leaf taken	Air space area
Vertical leaf	100 sq. mm.	12.4 sq. mm.
Horizontal leaf	100 "	28.1 "

These numbers represent the average of measurements taken from the tissue between the lateral vascular bundles, and in the

neighbourhood of the lateral vascular bundles, therefore the difference between the two is not so large as it would be, if the measurements were taken only from between the vascular bundles.

In both cases the mesophyll contains numerous oil canals. A consideration of the anatomy of both types of leaves leads to the conclusion that their structure shows modifications in connection with the process of transpiration.

In the case of the horizontal leaf the very thick coating of wax, particularly on the under surface where the stomata are situated, serves as a very effective means of diminution of transpiration (Briosi (5)).

The vertical leaf, though not provided with this heavy coating of wax, has, on the other hand, a very much thicker cuticle, which answers the same purpose. Since the enlargement and modification of the cells of the mesophyll surrounding the air space under a stoma coincide with a considerable diminution in the contents of the guard cells, it seems probable that they are so modified to regulate the amount of transpiration in the vertical leaf.

The air space area is less in the case of the vertical leaf than in the horizontal one, but the area of the vascular elements is considerably greater, and therefore the volume of water carried will be correspondingly larger.

Though there is very little difference numerically between the stomata of the two types of leaves, there is a noticeable difference in their size, those of the vertical leaf being almost twice as large as those of the horizontal. This is correlated with the greater vascular area, and appears to indicate that the vertical leaf may transpire more than the horizontal.

During the months of May, June and July, experiments were carried out with the two types of leaves in order to discover whether there is any difference in the rate of transpiration between the two. The apparatus used was the potometer. The horizontal tubes were of the same bore and divided into ten equal parts, each part being again divided into ten. Readings were taken at stated intervals of time, and the temperature recorded by means of the wet and dry bulb thermometers. The readings extended over varying periods of time from 10 a.m. to 8 p.m. for one or two days.

When the experiments were concluded, the area of the leaves was found in each case, and all the readings standardised by finding how many units on the horizontal tube 100 sq. cm. of leaf surface transpired in one minute. By this means it was possible to draw

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comparisons between the rates of transpiration of the different types of leaves, between the same times and under the same sets of conditions.

Unfortunately the supply of material was limited, and therefore my conclusions are based on a comparatively small number of experiments. The results obtained, however, did point to the vertical leaf transpiring more rapidly than the horizontal, as is shown by the two following examples:

EXAMPLE I. *In temperate house*

Rate per minute per 100 sq. cm. of leaf surface

No. of reading	Vertical leaf	Horizontal leaf	Remarks
1	—	·122	Range of temperature:
2	·096	·114	Dry 73-85.5°
3	·155	·147	Wet 71.5-82°
4	·218	·168	Weather: Sun
5	·218	·209	Dull from period 6
6	·101	·077	Range of time:
7	·070	·079	12.21 a.m.-7.50 p.m.
8	·036	·040	
9	·021	·025	
	·1143	·1073	Average rate for periods 2-9

EXAMPLE II. *In dark room*

1	·014	·0090	Range of temperature:
2	·013	·0102	Dry 56.6-57°
3	·012	·0099	Wet 56-56.5°
4	·013	·0102	Weather: Rain-heavy
			Range of time:
			2.5 p.m.-8.8 p.m.
	·013	·0098	Average rate for the period

CONCLUSIONS

1. Both types of leaves show structural arrangements which lessen transpiration.
2. The thick coating of wax on the horizontal leaves lessens transpiration, and at the same time prevents the wetting of the leaves.
3. The vertical leaf has no wax to lessen transpiration, but is possessed of a thick cuticle, which serves a similar purpose.
4. The vertical leaf possesses a special apparatus for the closing of the stomata. This is connected with a degeneration of the guard cells.

5. The larger vascular supply, and the larger size of the stomata of the vertical leaf, point to this leaf transpiring more rapidly than the horizontal leaf.

6. The experiments performed support the above statement. This has not generally been considered to be the case.

7. The greater development of the lens-like thickenings in the vertical leaf does not support Haberlandt's view that they are organs for the perception of light, leading to an orientation of the leaf, but rather points to their use for refracting the light on to the mesophyll cells below.

In conclusion I should like to thank Professor Weiss for his many valuable criticisms and suggestions during the course of the work.

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THE NEW PHYTOLOGIST

VOL. XXV, No. 4

30 OCTOBER, 1926

LIGHT AND GROWTH

III. AN INTERPRETATION OF PHOTOTROPIC GROWTH CURVATURES

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INTRODUCTION

IN the early days of plant physiology, tropic curvatures were interpreted as the direct result of the action of asymmetrically applied external agents upon the processes of growth, these processes being so disturbed that changes in direction of growth resulted.

Thus in 1806 T. A. Knight⁽¹²⁾, when describing his classical experiments with the apparatus now known as "Knight's Wheel," suggested that roots grew downwards because growth was restricted to the apex of the root, to which food gravitated, and which therefore only received adequate supplies of food when growing downward. Shoots on the other hand turned upwards, according to Knight, because growth of the shoot was not confined to the apex but resulted from extension of the fibres and vessels of the shoot. When the shoot was placed upon its side, the accumulation of sap upon the lower side produced a greater extension of this side and the re-erection of the shoot apex.

A similarly direct action of gravity upon growth was assumed by Hofmeister⁽¹¹⁾ and even by Ciesielski⁽⁸⁾, although the experiment first performed by the latter, in which the disappearance of geotropic

response is shown to follow upon the removal of the root apex, forms an important experimental prop to various theories which have since replaced these earlier views.

Similarly, in connection with phototropic response, de Candolle (7) suggested that the positive phototropic response of the etiolated shoot under lateral illumination was due to retardation in growth of the more brightly illuminated side. But in the last fifty years, particularly since Pfeffer's extensive publications (14-16), the view that tropic curvatures might be the result of the external agent acting directly upon the growing region, has receded almost completely into the background. Except for the conception of phototropism advanced by Blaauw (2), these external agents, in so far as they modify the direction of growth of plants, are almost universally spoken of as "stimuli" and are regarded as acting indirectly.

Pfeffer defines such a stimulus as a "releasing mechanism." Its action is indirect in the sense that there need be no quantitative relation between the energy applied externally and the resultant changes produced in the plant. The obvious analogy is the trigger action which detonates an explosive and releases its stored energy. The amount of energy released depends then upon the quantity and nature of the explosive and not upon the strength of the external force applied to the trigger. This appeared the only way to explain certain experimental facts, which were emphasised particularly in the "Power of Movement in Plants" (10), in which tropic response seemed to depend upon the perception of the stimulus by a localised region of the plant, *which was not the region of curvature*. For the experiments of Darwin confirmed Ciesielski, and led to the view that the root apex was especially sensitive to gravity although a region further back from the apex performed the geotropic curvature. Similarly Darwin, confirmed subsequently by Rothert (21), described experiments which demonstrated that the coleoptile of the Gramineae *curved from its base* towards the light when the apex alone was laterally illuminated. From Pfeffer's standpoint these experimental results were readily explained as due to the localisation of a special sensitivity of perception in these apical regions, coupled with a capacity to conduct the tropic "excitation" thus aroused to the growing regions in which tropic curvature then took place.

This standpoint also permitted a ready registration of almost innumerable facts as to the direction of growth of shoot and root systems and of the plant thallus in general, and for a number of years facts have been collected and recorded in terms of this theory

of "stimulus and response." The phraseology is largely based upon animal physiology, where more specialised mechanisms for the perception of light and gravity can be recognised and studied. Naturally, therefore, the more extended experimental study of the physiological processes involved, tended to assume the general truth of this view as to tropic mechanisms and to concern itself with such problems as the "threshold of response," i.e. the strength or duration of stimulus necessary to produce an excitation; the presentation time, i.e. the length of time for which a stimulus has to persist in order that a response may ultimately follow; the reaction time, i.e. the length of time after which such a response is visible, and the method of "conduction" of the stimulus from the "perceptive" to the "responding" region.

It should be realised, however, that the tropic machinery invoked in this theory remains hypothetical. Apart from the necessity for some theory, on which to generalise statements about such numerous phenomena, the indirect stimulus theory replaced the older standpoint of a more direct action of the external agent upon growth, mainly because the stimulus hypothesis permitted simple verbal expression (1) of the different tropic responses of different growing organs, notably shoot and root, and (2) of the response of a growing region when the stimulus was exerted upon a differently localised (perceptive) region.

It is the object of the present paper to show that in the case of the different phototropic responses of shoot and root, the older standpoint, represented by de Candolle, still supplies an adequate explanation of the phenomena. It is recognised, however, that this standpoint cannot be taken up solely in regard to one isolated series of tropic phenomena.

The coleoptile of the grass provides the classic example, in the case of phototropic response, of localisation of perception, and response in different regions of the plant. This case will therefore be examined fully in the next paper in this series. Subsequently it is hoped in a later series of papers, to re-examine the phenomena of geotropism from a standpoint which similarly excludes interpretation in terms of stimulation, and its perception with consequent excitation, transmission and response.

THE PHOTOTROPIC RESPONSE OF THE SHOOT

The Etiolated Shoot

Within the last twenty years Blaauw (2-6) has published a series of papers in which he has consistently advocated the interpretation of phototropic response in terms of the direct action of unilateral light upon the growing organ.

His experimental work demonstrated in the first place (3), that in the case of etiolated coleoptiles of *Avena sativa*, exposed to a light of steady intensity, exposure must be continued for a definite minimal period of time or no phototropic response will be observed.

But, conversely, if the duration of exposure is sufficiently long, any intensity of light may produce a phototropic response. The product of minimal time of exposure necessary into intensity of light always gave the same value of 20 metre candle seconds, below which light quantity no response *can be observed*. Thus there is no absolute threshold value of duration of exposure or of intensity of light. Experimental limitations prevented recognition of response in reply to light quantities smaller than 20 M.C.S., but there is a clear indication that with increasing quantities of light above this value, up to about 400 M.C.S., a larger light quantity produces a greater phototropic response. It has been customary to measure the light falling upon the plants during phototropic experiments by photometric methods, i.e. the light is measured by the effect it produces upon the eye. This is not a measure of the energy content of the light, but it is justified as a method of measurement by Blaauw's experimental demonstration that the curve of phototropic effect with wave length follows very closely the curve of visual efficiency.

Blaauw's experimental results indicate that there is a much closer quantitative relation between the quantity of light falling upon the plant and the phototropic response produced than is to be expected from Pfeffer's standpoint that light acts merely as a releasing stimulus. Blaauw is thus led to suggest that there is a close analogy between the response of the etiolated coleoptile to light and the photo-chemical effect produced upon a photographic plate by exposure to light. The etiolated coleoptile is, however, a very difficult case of phototropic response, and Blaauw's example will be followed in this paper in taking first, for further analysis, a less complicated case of phototropic response where the region which is illuminated is necessarily the region in which tropic response occurs.

Blaauw thus turns in a later paper (5) to the etiolated hypocotyl of *Helianthus*, and demonstrates that a definite light quantity falling upon it from all sides produces a definite effect upon the rate of growth. In these experiments he found that, after a brief light exposure, the growth rate of the hypocotyl, previously steady, has changed. At first it falls, to rise later, in some cases beyond its original value, to which it then subsides once more. But if the total growth over the period of changing rate is considered, this is always less than it would have been had the hypocotyl grown on uninterruptedly in darkness. From 4 m.c.s. upwards a fall in growth rate is observed and the effect increases with increasing quantities of light, but there is no simple relation between the amount of incident light and the extent of the reaction. Blaauw then showed experimentally that similar quantities of light, applied unilaterally, produced phototropic curvature, the tropic effect increasing with the increasing light quantities in a manner roughly proportional to the change in growth rate produced by increasing uniformly distributed illumination. Experimental proof was thus supplied that the direct effect of the unilateral light upon the growth rate is responsible for the curvature, the side exposed to the light growing more slowly and thus being on the inside of the phototropic curvature. The effect is obviously connected with the quantity of light falling on the plant, and Blaauw assumes that the photo-chemical action of the light is responsible.

The observation recorded in the previous papers (17, 18), in this series, seem to supply the strongest support to Blaauw's views, and indeed to give them actuality, since they show how the photo-chemical action of the light influences the growth rate of the etiolated organ. Such light quantities as are employed by Blaauw will produce a photo-chemical action in the shoot which will be especially pronounced upon the substances found at the surface of the protoplasts and intimately held in the intervening walls. As a result, fatty and protein substances will be released from complex forms of chemical combination; the proteins will disappear from the walls and the fatty substances migrate mainly to the cuticle. The walls of the cells of the meristem and of the tissues which intervene between the meristem and the vascular supply, are thus partly freed from protein and fat and now consist mainly of carbohydrates.

Along the walls the sap from the vascular supply can now percolate more freely and water soluble solutions in this sap find readier access to the superficial cells of the meristem, which are furthest away from the vascular supply, and which before were only growing

slowly. Increased superficial growth now ensues. Growth as a whole may be as active as ever on the more brightly lit side of the etiolated shoot, *but it is differently distributed.*

More cells are added to the surface of stem and leaf and less proportionately contributed to the inner layers of the shoot axis. The result is, therefore, in the aggregate, a retardation of growth *in length* on the illuminated side and a positive phototropic curvature.

As the light quantity falling upon the etiolated plant increases, so long as increased photo-chemical effect continues still to clear proteins and fatty substances out of the walls, the redistribution of growth becomes more marked and the phototropic effect, as also Blaauw's "photo-growth" reaction, increases. But there are obviously limits set to this process, because in time all proteins and fats which are still mobile will have been removed from the wall, and beyond this point increasing quantities of light will lose effectiveness. It is also intelligible from this point of view that the correspondence between quantity of light and rate of growth is not a simple one, as the light acts upon the distribution of substances in the walls of a complicated tissue system with, as a result, a redistribution of the growth activities within that system.

The Normal Shoot

The explanation given in the previous sections of certain positive phototropic curvatures obviously only applies to *etiolated* shoot systems. The point is not usually emphasised, but reference to any general account of phototropic phenomena will show that in fact the etiolated shoot is far more sensitive to light than any other type of shoot system. Whilst a few metre candles is sufficient light strength to produce phototropic curvature in the etiolated coleoptile or hypocotyl, prolonged exposures to ordinary daylight are usually required for the production of a phototropic curvature in the normal shoot system which has grown in the light. The light quantities involved are, therefore, of a totally different order of magnitude, and the phenomenon the result of quite a different train of internal circumstances associated with the conditions of development. This is to be expected because infinitely less light than that now required to produce curvature will have removed all the conditions characterising growth in darkness and will have set free from the network of walls between vascular system and meristem all substances which can be released as the result of photo-chemical action.

The limited range of these photo-chemical alterations in the walls of the tissues also explains Wiesner's (22) conception of "photo-mechanical induction," a change which results from lateral lighting and which finally ends in curvature, but a change upon which subsequent further exposure to light produced no effect in his experiments, modifying neither the extent nor the rapidity of the curvature. This photo-mechanical induction is now seen to be the completion of the photo-chemical change in the walls of the tissue in the side facing the light; maximum superficial growth and retardation of growth in length is then produced and subsequent further lighting is without further effect.

In studying the effect of various light quantities upon plants that were originally grown in darkness, it is obvious that a zone of transition will be met with where the phototropic action of the light passes from that characteristic of the etiolated plant to that characteristic of the normal shoot.

In this transition experimental region the effects of the light may be very difficult to interpret, as is the case with many experiments recorded by Pringsheim (20), Clark (9), and Arisz (11). As, however, nearly all these experiments were carried out upon the coleoptile of *Avena* their consideration is deferred until the phototropic mechanism of this organ is considered in detail.

Pringsheim (20) also carried out less extensive experiments with etiolated dicotyledonous seedlings which showed that whilst the etiolated seedlings responded to his light source at a distance of 100 centimetres within a definite period of time, when at a distance of only 10 centimetres from the light, they reacted much more slowly. This contradiction is now explicable. With the light one hundred times stronger the restrictions to sap flow and meristem nutrition, imposed by etiolation, soon disappear throughout the seedling, and the positive phototropic curvature of the etiolated seedling detected with the weaker light is not seen in the strong light. On the other hand, seedlings previously grown in the light, only responded to the lateral illumination when placed relatively close to it; at a distance like 100 centimetres no phototropic curvature was obtained, nor was it to be expected, as these seedlings had not the reactivity of etiolated seedlings.

At present it is only possible to interpret on quite general lines the phototropic curvatures which occur frequently, but by no means invariably, when normal light-grown shoot systems are exposed to incident lateral light. Growth of such shoot systems is the result

of the activity of apical and intercalary meristems⁽¹⁹⁾. The activity of the meristem is dependent upon a supply of sap from the vascular system of the plant and during the sunny hours of the day it frequently happens that such a sap supply is deficient and growth ceases. In this delicately balanced equilibrium, *strong* lateral illumination may mean that the sap supply first fails on the side more directly lit, where evaporation will more rapidly bring about that state of "incipient drying" in the walls of the tissues which Livingston⁽¹³⁾ has demonstrated as a frequent phenomenon in the cell walls of the green leaf. If the walls between the vascular supply and superficial meristem are in this condition, food supplies to the meristem will fail, and there will be a cessation of meristematic growth and also of extension of differentiated tissues as the result of vacuolation.

This condition prevails first and is less readily removed by increasing sap supply upon the more illuminated and, therefore, drier side of the shoot. The result may well be a positive phototropic curvature and the habit of growth of many plants in well lighted windows seems thus adequately accounted for.

Many cases of phototropic curvature, such as the complex movements of inflorescences and flower pedicels, will require much closer analysis from this standpoint; in many cases gravity also plays a rôle. Further elucidation of those phenomena from this standpoint must wait upon detailed experimental study of individual cases. It is the purpose of this brief section to show that an interpretation of phototropic phenomena from the new standpoint is possible, and that it is essential to emphasise the clear distinction which must be drawn between the phototropic response of the etiolated and the normal shoot.

THE PHOTOTROPIC RESPONSE OF THE UNICELLULAR HYPHA

Before proceeding to consider the case of the root it is necessary to state very briefly the results obtained by Blaauw in an extensive study of the light-growth reaction of the sporangiophore of *Phycomyces*⁽⁴⁾.

This erect unicellular hypha is very sensitive to light. When illuminated from all sides by a system of mirrors a light quantity of 210 M.C.S. (14 metre candles applied for fifteen seconds) produced no result for from 2-4 minutes, then the growth rate is accelerated, reaches its highest point in from 6-8 minutes (a rate from two to two-and-a-half times the normal), then falls to about 75 per cent. of its original value, returning to the normal rate again at the end

of about 14 minutes. The aggregate result of this light is thus a considerable increase in the amount of growth made in the subsequent quarter of an hour.

This is at first sight rather puzzling, because light quantities of the same order of magnitude falling upon the hypha from one side only produce *positive* phototropic curvatures. It must be remembered, however, that we are dealing with a single cell, a transparent cylinder full of aqueous sap, and brief consideration will show that when weak unilateral light falls upon this, the lens action of this cylinder, circular in cross section, will produce a concentration of light upon the *wall* on the side away from the light. In this case the difference in illumination is experienced in the wall, and the growth rate is determined, not by protoplasmic increase but by cell elongation, due either to increased osmotic pressure of the cell sap or an increase in the extensibility of the wall. A greater illumination of the wall may readily modify the extensibility of the wall, and the results of uniform illumination show that this result must be in the direction of diminishing its resistance to stretching.

When lateral light is concentrated on the wall distant from the light, therefore, this side extends in length more than the other, and the turgid hypha bends in the direction of the light source. Blaauw's experimental data adequately support his explanation of the behaviour of this unicellular hypha, and it will be noticed that the mechanism at work is again photo-chemical action of the light at the surface of protoplast and walls. In this case the result is a greater extensibility of the wall which consequently stretches under the hydrostatic pressure of the cell contents.

The sporangiophore when not illuminated is growing in a dull red light to which it is insensitive so that this is again a case of the response of a sensitive etiolated organ. If the light quantities exceed about 200 M.C.S., the growth reaction and the curvature both begin to disappear, and with much higher light quantities, if the light is applied with sufficiently high intensity, new and different reactions appear, varying in individual cases, but often showing indications of negative curvature. These are probably to be connected with differential drying of the hyphal wall, the drier wall being naturally more resistant to stretching. These results are obviously in harmony with those obtained with the shoot; they may also throw considerable light upon the difficult problem of the phototropic curvature of the root.

THE PHOTOTROPIC RESPONSE OF THE ROOT

In the root the meristem is not superficial, but forms a lens-like mass of tissue which is buried in the apex and caps the end of the endodermal cylinder (20). If then the effect of light in producing phototropic curvature depends upon photo-chemical action upon walls intervening between the centrally placed vascular system and the meristem, it is not surprising to find that the great majority of roots show no phototropic curvatures.

Blaauw (6) has shown further that when the growth rates of the phototropically insensitive roots of *Lepidium*, *Raphanus*, and *Avena* are measured, no difference is found when the root is transferred from darkness to uniform illumination.

Difficulties arise, however, in the interpretation of the negative phototropic curvatures of light sensitive roots. The only case intensively studied by Blaauw is that of *Sinapis alba*; in this case uniform illumination, if of long duration, produces a retardation of growth rate. It is noticeable that whilst a brief light exposure to about 150,000 M.C.S. will produce a slight growth retardation subsequently in the phototropically insensitive roots of *Lepidium sativum*, a short exposure to 300,000 M.C.S. will not produce a recognisable effect upon the growth rate of *Sinapis*. On the other hand a long exposure to 64 metre candles is much more effective upon *Sinapis* than *Lepidium*, this effect increasing with *Sinapis* with higher intensities whilst it is no longer recognisable with *Lepidium* with intensities of 500 M.C.

Long exposures to unilateral light intensities of 64 M.C., and over, also produces quite definite negative phototropic curvatures, although uniform illumination retards growth. Blaauw argues very plausibly that the same type of explanation may be applied to the apparent contradiction as in the case of the unicellular hypha. He points out that the curvature appears to be produced as the result of a difference in growth rate in the region within two millimetres of the extreme apex, and that so long as the region is laterally illuminated the phototropic effect is obtained. The root of *Sinapis* is very slender and transparent, the density of this apical region being somewhat different from the rest and he illustrates photographically an apparent concentration of the light by refraction in the side away from a lateral light source. This concentration of the light upon the distant side will then be followed, to judge from the effect of light upon growth rate, by a retardation of growth on the side of the root away from the light, and thus a negative phototropic curvature.

It is difficult, however, to accept this explanation. In the first place the concentration of light by refraction on the side away from the light source will not have so important an effect upon the distribution of the light as the scattering due to the heterogeneity of the tissues and the cell contents.

The latter effect will, however, have as a result a growing loss of light as it passes through the tissues from the illuminated side. This effect, even in a transparent thin root like *Sinapis*, will certainly be very marked, and in the relatively massive aerial roots of *Chlorophytum*, which show the same negative phototropism, there can be no doubt that the side towards the light is more strongly illuminated. Furthermore the phototropic response is not modified with either of the roots when they are laterally illuminated in water instead of in damp air, in spite of the difference in refractive index of air and water.

Blaauw describes some experiments in which the roots fail to curve when placed in paraffin oil, but as they almost fail to grow any more under these conditions this is not surprising. It is possible, however, to see an explanation of these results which is relatively simple and seems to be in accord with the experimental data.

The phototropic curvature, as Blaauw points out, does not occur throughout the whole region of root extension, but in the region just behind the apex. In this region the extension of meristematic cells into vacuolated cells is commencing, and the relative lengths of the sides towards and away from the light, depend on the rates at which the early extension of the two sides takes place. It would appear then, that in negatively phototropic roots like *Sinapis* and *Chlorophytum*, the extension proceeds more rapidly when the roots are illuminated, just as it does in the sporangiophore of *Phycomyces* (and as it will be found to do in the vacuolating cell of the coleoptile). As a result the cells of the tissue of the root, from the endodermis to the outermost layers of the cortex, are found on examination to be longer on the illuminated side in the region of the curvature. This is easily demonstrated by the study of a microtome section through the region of curvatures in the case of both the roots mentioned. This temporary increase in the rate of extension, at a particular stage in the differentiation of the cell is not accompanied by an increased rate of growth under uniform illumination, because it is followed by an extension to a final length which is on the average smaller than the average length of the cell extending completely in the dark. As, however, cell extension is accompanied by cell thickening, this

later general extension to a greater length is unable to correct the curvature first induced by lateral illumination; the tissues of the root being maintained at the angle of curvature by the rigidity given to the walls by the deposits that follow after the curvature has taken place. As a result the phototropic curvature cannot be removed by exposing the bent roots to chloroform, or other agencies which dispose of the turgidity of the protoplasts.

It is not easy by measuring the cell length of the tissue in microtome section to obtain an accurate comparison of the length of cells in roots grown in the light and in the dark. If, however, roots are thus grown in light and dark, or germinated in the dark and then transferred to the light, all roots being kept in a thermostat at a constant temperature, the roots in the dark are found to be some 20 per cent. longer than those grown in the light. Then, on cutting out short pieces of the root, at comparable regions, that is from the base where the cells are equally old and presumably have reached their maximum extension, it is possible by maceration, in alcoholic hydrochloric acid (25 per cent.) followed by 0.5 per cent. aqueous ammonium oxalate, to separate the endodermal layers by gentle tapping upon the cover-glass when the roots are mounted in glycerine, and to measure the length of the cells of this layer with great precision. The cell lengths are somewhat variable, but comparisons of the longest roots grown under such experimental conditions, leaves no doubt that these cells are longer in the roots grown in the dark.

In the light these cells seem, as the average of numerous countings, to be some 20 per cent. shorter than in the dark. Some difference between the rate of growth in the light and dark might be due to differences in meristematic activity, but there is no sign of any such difference in microtome preparations, and as the average differences in cell length appear to be about the same as the differences in root length, the shorter cells formed in the root in the light probably account completely for the slower rate of growth in the light.

SUMMARY

1. Blaauw explains the positive phototropic response of the etiolated shoot as the result of the slower growth of the side towards the light.

2. The previous studies in etiolation show that this slower growth under illumination is to be explained as the result of a redistribution of growth. The photo-chemical action of the light releases protein and fatty substances from the walls of the cells intervening between

vascular bundle and meristem; as a result the meristem cells at the surface of the shoot are able to grow freely and a more superficial tissue growth takes place, instead of the almost exclusive growth in length which occurs in the etiolated shoot.

3. The light quantities required to produce phototropic curvatures in an etiolated shoot are very small, as are the light quantities required to modify its rate of growth.

4. The normal light-grown shoot is much less sensitive to light. Phototropic curvature, if it occurs, can only be induced by prolonged and strong light exposure.

5. The possible effect of such strong lateral light upon the growth of the normal shoot is discussed. Such light may induce phototropic curvature by its influence upon the nutrition and extension of the cells at the growing point. This phototropic mechanism must be sharply distinguished from that operating in the etiolated shoot.

6. Blaauw's experimental results upon the phototropic curvature of the sporangiophore of *Phycomyces* are briefly recapitulated.

Light is concentrated by lens action upon the side of the wall distant from the laterally placed light source, this side therefore becomes more extensible as the result of photo-chemical changes, and its greater extension leads to a positive phototropic curvature.

7. In the light of these experiments a new explanation of the negative phototropic curvature of light-sensitive roots is advanced. It is attributed to the quicker extension of cells which are just commencing to vacuolate as the result of their stronger illumination.

8. On the other hand, the slower rate of growth of these roots in the light is explained as due to the fact that in the light the cells of endodermis and cortex finally attain a shorter adult length.

9. Most roots are not sensitive to light. The difference between shoots and roots in this respect follows naturally from the different disposition of the meristematic tissues at the growing points.

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LIGHT AND GROWTH

IV.

AN EXAMINATION OF THE PHOTOTROPIC MECHANISM
CONCERNED IN THE CURVATURE OF COLEOPTILES
OF THE GRAMINEAE

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(With 4 figures in the text)

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INTRODUCTION

THE new interpretation of the changes effected by light on the development of the etiolated plant given in the earlier papers (22-24) showed clearly that unilateral light, by bringing about a redistribution of growth on one side of the plant, must cause a positive phototropic curvature. The adequacy of this explanation has been tested for the etiolated shoot and negatively phototropic roots, and the present paper is reserved for the re-examination of the case of the etiolated coleoptile of the grass seedling, where a mass of experimental data has been accumulated in support of an entirely opposite view.

Darwin (13) first pointed out that if light falls laterally upon the apex, the curvature of the coleoptile, which takes place towards the light, is wholly brought about by a change in relative length of the tissues of the unilluminated region. This has invariably been explained as the result of the "reception" of a directive "stimulus" by the apex, which is consequently "excited," the stimulus is then

transmitted to the region of curvature, and phototropic response follows.

If this interpretation of the phenomena is correct, the explanation of phototropic curvatures, given in the first paragraph, is under suspicion, as it is unlikely that entirely different mechanisms are at work in the etiolated shoot and in the etiolated coleoptile. As a result, however, of a reconsideration of the data, it seems possible to conclude that an explanation of the behaviour of the coleoptile on the lines of the direct action of light upon growth, meets with fewer difficulties and inconsistencies than an interpretation in terms of the classic theory of stimulus, excitation, transmission and response.

The very numerous experimental data on the phototropism of the coleoptile will first be briefly recorded from the observers' standpoint; in a second section the data will be re-examined as relevant to a somewhat wider field of enquiry, more closely concerned with the phenomena of growth and differentiation. It will become evident that the phototropism of the coleoptile can be much better understood when details of its structure and development are considered and on these questions some additional data will be published later. For the purposes of the first section it is sufficient to recall that the coleoptile is a hollow cylindrical organ with a solid pointed apex, which grows up as part of the plumule of the grass seedling. It contains within it the first normal leaf, which is rolled upon itself. The coleoptile is of limited growth, at a certain stage the leaf within breaks through the coleoptile which remains as a sheath around its base. The etiolated coleoptile is extremely light sensitive, the leaf within is quite insensitive (Paál (20)) to lateral light, but the hypocotyl or mesocotyl if present also exhibits phototropic curvature.

The coleoptile in transverse section is oval to oblong. The two veins run up to the apex through the tissue of the two narrow sides. One of the broader flanks is pressed against the scutellum. No further details of structure seem necessary at this stage, but a fuller discussion of the nature and structure of this organ will be found at the commencement of the second section.

I. EXPERIMENTS ON THE TRANSMISSION OF PHOTOTROPIC STIMULI FROM THE APEX OF THE COLEOPTILE

Since the time of Charles Darwin (13) it has been known that lateral illumination of the apex of the coleoptile alone is followed by positive phototropic curvature, the movement being brought about by unequal extension of the base of the organ.

These earlier data of Darwin on *Phalaris* were confirmed by Rothert in a very extensive series of observations on *Avena* (31). In the Paniceae, Rothert concluded that the coleoptile alone was sensitive to the actual light stimulus, though the hypocotyl might perform phototropic curvatures as the result of the illumination of the coleoptile. Pisek (21) and Sierp and Seybold (38) in very recent papers, supply more precise experimental data which confirm the general view that the apex is very much more sensitive to light than the rest of the coleoptile. The latter authors show that this sensitive region is very short, not more than two millimetres in *Avena sativa*.

Rothert was, apparently, the first author to show that after cutting the veins, the stimulated tip of the coleoptile could still transmit the excitation through the ground parenchyma. Fitting (14) confirmed this observation in a series of elaborate experiments and arrived at the rather difficult conception that a state of polarity is induced by the light stimulus and transmitted throughout the plant by living channels, independent of the vascular bundles.

Boysen-Jensen had a rather different experience in his experiments with etiolated coleoptiles (5, 6). Fitting had kept his plants in a saturated atmosphere. Boysen-Jensen tried to repeat his experiments with plants growing in ordinary laboratory air. He then found that if the vein was cut on the side away from the light, no tropic curvature took place when the apex was laterally illuminated; on the other hand, if he cut the vein on the side towards the light, the usual curvature followed as the result of unequal growth of the two sides of the coleoptile below the region of the cut. Similar results were obtained if the cut region of the coleoptile was under water, but if the coleoptiles were kept in saturated air, Fitting's experimental result—that both veins could be severed and the stimulus still transmitted—was confirmed.

Boysen-Jensen concludes that in a saturated atmosphere, the cut is kept full of sap and any substance diffusing from the apex can pass; when the cuts are under water presumably this substance diffuses away and is lost. The different results of cutting different

veins in the experiment in dry air suggests that transmission on the side on which greatest extension is to take place is more important and therefore that the substance transmitted is a substance that promotes growth. A similar conclusion is supported by the experiment made by both Fitting and Boysen-Jensen in which a mica plate was inserted in the cut. Such a plate only prevented curvature when inserted between the ends of the vein on the side away from the light. Snow (39) agrees that curvatures are still obtained when a mica plate is inserted in a cut on the illuminated side, and experiments by Purdy (28) and Nielsen (19) also support the view that growth-promoting substances are travelling from the apex along the side away from the light. Boysen-Jensen then tried the experiment which has so captured the imagination of plant physiologists generally. He tried the effect of cutting off a centimetre of the apex of the coleoptile which was then replaced upon the stump with the aid of just melted gelatin, and the ring join sealed with cocoa butter. When the tip alone was now laterally illuminated phototropic bending of the basal region still occurs. The stimulus can thus be transmitted apparently across a layer of gelatin. Naturally such a suggestive experiment has already been frequently repeated, although this first experiment is relatively recent (1910), and as a result quite a technique has developed for cutting off and replacing the apex. This subject had, therefore, better be specially considered.

The experiment is not without difficulty. Many of the observers report trouble through the washing off of the tip owing to guttation (Paál (20), Söding (40) and Snow (39)); on the other hand if the plants are kept too dry the apex wilts (Snow (39)). Boysen-Jensen later discarded the superficial dressing of cocoa butter as unnecessary, gelatin has usually been employed to cement the tip to the stump, though it will often remain on without any adhesive (Stark (41)). The internally contained first leaf is obviously a difficulty; it grows up and pushes the tip off again in many cases. Paál got rid of this trouble and of guttation by removing the coleoptile from the seedling by a ring cut at the base, and planting the hollow coleoptile by itself in wet sand. Needless to say the growth responses of such separate coleoptiles were much smaller and they had to be collected at a fairly early period of their growth. Stark and Drechsel got rid of the first leaf, as usually it is possible to break it across at the base and then pull it out (42).

Paál and Stark tried to replace Boysen-Jensen's transverse cut across the coleoptile by two cuts meeting in an angle (Text-fig. 1),

but although this enables a more exact replacement of the apex from where it was originally taken the result is not satisfactory; the traumatopic effect of the two cuts is not adequately balanced and irregular curvatures often result. Stark finally adopted the plan, which Snow also finds successful, of cutting the coleoptile on one side and then breaking it across, the break being perfectly flat.

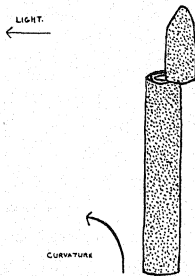
Paál's extensive and careful work drew much more attention to the general significance of this experimental method. His control experiments seemed to place beyond all doubt the fact that the curvature of the base was not the result of stray light reaching it from the illuminated apex. Thus, to cite only one experimental



Text-fig. 1



Text-fig. 2



Text-fig. 3

control, the cut apex was placed on top of an uninjured coleoptile (Text-fig. 2); if then stray light from the illuminated apex was responsible, curvature of the uninjured coleoptile below should follow, but it did not take place. In Paál's experiments, and indeed, in most experiments, a narrow flank of the coleoptile is turned towards the light; tropic curvatures are then more regular than when a broad surface is towards the light; in such cases the identification of a phototropic curvature may be rendered more difficult on account of spontaneous curvatures of the nature of nutations which are referred to again on p. 238. Paál sometimes separated the apex from the stump by a thin slice of the porous tissue of the stem of *Calamus*, which was soaked in gelatin, and still in 74 out of 91 cases obtained a tropic response in the stump.

If, on the other hand, the apex was separated from the stump by mica, by cocoa butter or by platinum foil, no response of the base took place.

A further experiment by Paál leads to complications in the interpretation of these experiments. In *Coix*, which has a large coleoptile, he cut off the apex and placed it back, without gelatin, as in the Text-fig. 3, in the dark. The result was a curvature through 90° with the side of the stump beneath the tip on the outer convex side of the curve.

On the other hand, if the tip be replaced *with gelatin*, no curvature takes place. Paál concludes that, therefore, there must be two types of substances diffusing from the apex. The one kind, promoting growth, cannot diffuse through gelatin; the other, arising as a result of light stimulus, can diffuse through gelatin and control phototropic curvature of the stump. These controlling substances Paál, like Boysen-Jensen, also considers to be growth promoting, but whilst Boysen-Jensen thinks that they are moving down in greater quantity on the side away from the light as the result of the stimulus, Paál considers that they are either partially destroyed on the lighted side as the result of the illumination, or else, in some manner, partially prevented from passing. Thus Paál pictures the curvature as due to lesser growth on the side towards the light, Boysen-Jensen as caused by greater growth on the side away from the light(7).

Stark(41) attempted to get further light upon the nature of these substances controlling tropic curvature by experiments in which the apex was replaced either on its own stump, or the stump of another plant of the same species, or upon the stump of a plant of another species with, of course, a varying degree of relationship. Stark thus found(42) that a stump, crowned with a foreign apex, continued to respond phototropically when the apex was illuminated, though usually much more weakly; but if to a *Hordeum* stump the relatively more sensitive *Avena* apex is attached, the resultant phototropic curvature in the base may be more pronounced than usual. Stark had already noted(41) in connection with the transmission of traumotropic stimuli across the junction from stimulated tip to responsive coleoptile stump that it did not matter in the least whether the apex was replaced accurately in position, it could be placed back to front, or in any practicable position without disconcerting the directive response of the stump. His new experiments bring him to the somewhat difficult conception that, whilst the result of the stimulus must

be a growth-regulating stimulus substance, the substance must be specific, and at the same time between the substances formed in the apices of allied species a somewhat similar generic chemical resemblance must exist. And all these substances, it must be remembered, have to diffuse through gelatin!

Stark and Drechsel⁽⁴²⁾ added one exceedingly interesting experiment; they took apices which had been laterally illuminated from one side, cut them off and placed them on stumps of coleoptiles which were grown in the dark, decapitated, and still kept in the dark. Curvatures now take place, which are still in the positive phototropic direction in reference to the previously illuminated tips.

Brauner⁽⁸⁾ has carried out the reverse experiment to this. The points of coleoptiles were removed and kept in the dark and the stumps exposed to lateral light. Then the points were very carefully replaced on some of the stumps, and all the plants kept in the dark for four hours. Out of 32 with points replaced 21 bent in the original light direction, whilst those without points continued to grow straight.

Finally, a few experiments must be referred to which are directed to elucidate the nature of the growth effect produced by the substances diffusing from the apex. Stark has tried the effect of placing extracts from wounded coleoptiles on one side only of the coleoptile stump and finds as a result that the stump shows curvature towards the side thus stimulated. This suggests that the stimulating substance is retarding or diminishing growth on the stimulated side. Brauner concludes that the illumination of the apex of the coleoptile actually diminishes its growth from his experiment described above; he concludes, however, that the growth-retarding substances are not produced in the apex as the result of the light stimulus, but are always being produced there; when, therefore, the laterally illuminated stump receives its apex again, these substances can travel down the illuminated side more readily because of its greater permeability as the result of illumination, and thus phototropic curvature follows now in the dark.

Nielsen⁽¹⁹⁾ reaches a somewhat different conclusion. He agrees with Boysen-Jensen that growth-promoting substances travel from the apex and confirms Paál's experiment that when the apex is replaced on one side of the stump only, growth goes on more strongly on this side. If, however, rings of tissue from the middle or basal zones of the coleoptile are placed over one side of the cut end he finds, as also did Stark, that the stump curves towards the side

bearing this ring, as if the substances diffusing from it retarded growth. A ring of tissue taken from near the apex of the coleoptile behaves like an apex and seems to promote the growth of the flank of the stump on which it rests.

Nielsen, therefore, concludes that whilst growth-promoting substances are released from the apex, growth-retarding substances must be present in the base of the coleoptile.

When he tried the effect of expressed sap from the coleoptiles which he fed to one side of a decapitated stump by means of fine capillary tubes, then, whether he took the sap from tips or from whole coleoptiles, in every case it seemed to retard the growth of the side to which it was added.

Seubert⁽³⁵⁾ also carried out some experiments which were intended to elucidate the chemical nature of these substances, but can hardly be said to do so as they suggest that extracts from unstimulated coleoptiles, neutral salts, sugar, enzymes, etc., all produce the same type of curvature if applied laterally to a cut surface of the apex, and all produce the same growth response if applied to the cut stump, in each case being applied in agar jelly. If the solutions are weak the curvature is in one direction, and growth rate greater than in cut stumps untreated; in more concentrated solutions the curvature is in the other direction, and the growth rate smaller than that of the cut stump.

In concluding this section then, it may be permissible to point out what a pyramid of conceptions are struggling to maintain themselves upon the one general experimental fact—the phototropic response of a coleoptile stump when its severed apex is replaced and alone laterally illuminated.

Localisation of perception and response are thus assumed to be proved and transmission by definite growth-regulating substances regarded as rendered highly probable. These substances are still of a quite unknown nature; they are sometimes said to accelerate, sometimes to retard, growth; they diffuse through gelatin, and yet are required to have a complexity of structures enabling both specific and generic differences to be mirrored in their differences of chemical structure. They are usually assumed to be formed on stimulation, and yet other experiments suggest they must be always present and they have to be distinguished from other growth-promoting substances present in the apex, which do not diffuse through gelatin, and yet other growth-retarding substances assumed to be present at the base of the coleoptile. In the next section an attempt will be

made to place these experiments in focus in a wider field of data which includes both the facts of development and anatomy, and the very considerable data also available as to the growth and permeability of the coleoptile and the effect of light upon these associated phenomena.

II. THE MODE OF GROWTH OF THE COLEOPTILE AND THE EFFECT OF LIGHT UPON IT

The morphology of the coleoptile is still a subject of discussion (32, 33, 45). It develops as an outgrowth of the adaxial surface of the scutellum (Worsdell⁽⁴⁵⁾ reproduces figures of early stages from Celakovsky) and this has led to the view that it is to be regarded as a fused pair of ligular outgrowths, which bear the same relation to the scutellum or cotyledonary leaf that normal ligules bear to the ordinary leaf. It is true that the vascular strands to scutellum and coleoptile are sometimes separated at their insertion on the axis, by the length of the hypocotyl or mesocotyl, as in *Panicum*. But a comparison of this type with *Oryza* (both types are figured in Schlickum⁽³³⁾) in which the vascular bundles of scutellum and coleoptile arise at the same level, the strand to the scutellum then running downward through the mesocotyl, suggests that meristematic activity at the node has probably given rise to the mesocotyl. In this case it is simply a matter of the exact position at which the meristematic activity proceeds that determines whether the strands to scutellum and hypocotyl shall arise in the same horizontal plane or be separated in their insertion by the length of the mesocotyl.

Van Tieghem⁽⁴³⁾ emphasised this point of view when he spoke of the axis between insertion of coleoptile and scutellum as an elongated node. The peculiar nature of this part of the axis is indicated by the use of the term mesocotyl (Celakovsky) which does not assume its strict homology with a typical hypocotyl.

The coleoptile develops as a lobe on the scutellar side of the plumular axis and only later forms a fold all round the axis. The ring of tissue then grows up, fuses as a conical roof over the undeveloped leaf rudiments of the plumule, and thus forms the hollow structure which completely encloses the next leaf. This habit of growth has led to the view that the coleoptile should be regarded as a leaf sheath (with ligule) of a very undeveloped leaf—the scutellum. From our present point of view these two views are in agreement on what is the essential point, viz. that the coleoptile must be regarded as an organ, whether ligule or sheath, of which the growth is strictly

limited. The development of an organ of limited growth has not often been studied in detail, but in general it is almost certainly true of it, that after a certain period of meristematic growth, the cells of the structure *all* pass into the vacuolated condition and cease to grow, as has been seen to happen in the case of the leaf sheath of *Tradescantia* (34). When this tissue is thus all vacuolated no further possibility of growth persists, nor of growth movements.

A fuller report upon the development and anatomy of the coleoptile will be published later, but one very important point must be made now. In all the experimental work upon coleoptiles, seedlings are taken in which this organ is at least about half a centimetre long.

In such plants the meristem has already disappeared and growth of the coleoptiles under experimental conditions takes place through the vacuolation and extension in length of existing cells; no new cells are formed as the result of meristematic activity. The meristematic condition persists longest at the base and on the whole extension of the cells takes place first, and ceases first, from above downwards (Rotherth(31) noticed this basipetal extension in the case of the long coleoptile of Sorghum), but the mechanism of phototropic response must be entirely connected with cell extension, not with cell formation.

These considerations throw a flood of light upon Vögt's(44) careful observations upon the growth of the coleoptile and the manner in which this is influenced by light. Vögt found that the rate of growth of the coleoptile in the dark followed the curve typical of what Sachs termed a "grand period."

The rate rises to a maximum reached within a day or two, but not long maintained, and then falls finally to zero. If light falls upon the etiolated coleoptile for a short period of time, this produces a short and very slight drop in the growth rate, followed by a marked increase. The effect is the same, whether the light acts for a short time or a considerable period, and within wide limits, whatever the intensity. If, however, the light effect is studied not upon the growth rate but upon the final length of the coleoptile, then invariably, with increasing quantities of light (i.e. intensity \times time) the length of the adult coleoptile is proportionately less.

From the standpoint of development it is clear then that the *rate* of extension of the vacuolating cells is increased by illumination but the final length of the individual cells is less. This, it will be remembered, is exactly the effect of light which was used in the previous paper(24)

to interpret the phototropic effect of light upon the light-sensitive root. The original slight fall in growth rate is also very suggestive of the effect of light upon etiolated shoots previously reported (23), the tissues become more permeable and the sap more widely distributed. Such greater permeability in the coleoptile might lead to a temporary wider spread of the sap from the veins followed by temporary fall in the rate of vacuolation, immediately checked as more sap rises from the root system. It must be emphasised here that almost every experimental fact recorded as to the influence of light upon the etiolated shoot, the disappearance of the plumular hook, development of the capacity to plasmolyse, the loss of starch from the parenchyma, are arguments in support of the view that the access of light to the etiolated coleoptile will facilitate the entry of water into the cells. To these may be added the observation of Zollikofer (46) that in the coleoptile in the dark, starch disappearance begins at the apex and proceeds basipetally, though it is never complete. If the coleoptile is exposed to the light, starch disappears much more rapidly in a basipetal direction and may finally go altogether.

The freer movement of the sap in the walls of the coleoptile on illumination seems also to be the explanation of Brauner's (9) results. The electrical resistance across the coleoptile was measured before and during illumination, and its conductivity increased very considerably during illumination.

Brauner (8) also attempted to correlate the effect of uniform illumination upon the rate of growth of the coleoptile with the phototropic curvatures occurring under lateral illumination. Sierp (36) had already pointed out that these phenomena are more complicated than Vögt had observed. Sierp found, and Brauner confirmed, that the subsequent growth rate after illumination was not regular but passed through maximum and minimum phases. This is perhaps to be expected as the light is another factor superposed upon a growth rate that normally rises to a maximum. Usually now, however, a series of waves can be traced in the curve of growth rate, falling in amplitude. Sierp compared his data with phototropic responses observed by Arisz (1) and Clark (12), which similarly fluctuate between successive positive and negative responses following upon the original exposure to lateral light, and thought the agreement good. With small light quantities the agreement found by Brauner between the occurrence of minimum growth rate and maximum positive curvature is exceedingly good. With strong light of short duration the agreement

is still good but with long exposures correlation is poor unless the *apical region is shaded* from the light. On the whole the conclusion from these experiments is that, apart from special peculiarities connected with the effect of light upon the apex, there is close correspondence between the very complicated photo-growth reaction and the phototropic response. This conclusion has, however, often been called in question (Lundegardh (18), Von Guttenberg (15) and Burckhardt (11)); a good recent review of the controversy is given by Pisek (21). The discussion will not be taken further here, however, as the question takes on another aspect after the question of the special sensitivity of the apex has been considered.

The Apex of the Coleoptile in Relation to Light

The significance of the apex of the coleoptile in relation to normal growth first needs consideration. The pointed tip consists simply of nearly iso-diametric cells which have elongated very little during vacuolation, just beneath this tissue the two veins which run up the narrow flanks of the coleoptile come to an end. These veins, close below the apex, run obliquely outwards towards the broad flank of the coleoptile which faces away from the scutellum.

Each vein ends in a little pocket of short tracheids which come nearly to the surface of the coleoptile. The stomata in the outer epidermis, which are to be found lying above the route followed by the veins, are also to be found congregating in the region where these vein ends approach the surface. The result is that a little way below the apex, on the broad flank facing away from the scutellum, lie two, nearly confluent, potential hydathodes.

When guttation begins in the coleoptile, as it does very early in the development of the seedling, examination with a lens will show that the drop of sap exudes from these hydathodes. This will certainly mean a release of sap mainly from this flank of the coleoptile, and it is therefore of very great significance that autonomous "nutational" movements of the coleoptile are recorded by so many observers as occurring mainly in the direction of the short transverse axis (see p. 231).

These autonomous curvatures have undoubtedly led many earlier workers with the coleoptile into error. They have been studied recently in seedlings mounted in the klinostat, Lange (17) pointing out in this connection the advantages of an electrically drawn apparatus which, because of its large reserve of power (30) drives at the same speed through every quadrant of the circle. Lange con-

firms Bremerkamp's⁽¹⁰⁾ earlier observation that the autonomous movement of the coleoptile apex is outwards away from the scutellum, Pisek⁽²¹⁾ is in agreement, but none of these observers note the correlation between the *loss of sap* from this flank and the *curvature* towards it. It is submitted that, bearing in mind the fact that the whole length extension of the coleoptile is a question of cell extension as the result of intake of water, the connection between loss of sap from this flank and its diminished extension is casual. But all through the literature of the tropism of the coleoptile there is a running commentary on the activity of the apex in guttation. Renner⁽²⁹⁾ notes, in experiments in which the coleoptiles were kept in small glass tubes, and in which he again found growth-rate changes running inversely with curvature, that their guttation never ceased.

But if the permeability of the tissues is affected by light in the way demonstrated by Brauner⁽⁹⁾ then the special effect of the light upon the apex of the coleoptiles may result from the greater guttation from the flank exposed to the light; naturally therefore more uniform results will be obtained from the experiments in which one of the narrow flanks, *containing a vein*, is exposed to the light.

It is suggested, therefore, that this is the machinery which causes the basal curvature when the apex alone is exposed to the light. As the vascular system of the hypocotyl will be also in communication with the veins at the apex of the coleoptile it also is intelligible that the hypocotyl may show phototropic curvature as the result of the exposure of the coleoptile apex to light, whilst itself apparently uninfluenced by lateral lighting, save that the final length of the hypocotyl is shorter under uniform and fairly strong illumination (Rothert⁽³¹⁾, Von Guttenberg⁽¹⁵⁾).

Guttation has now to be taken into account in connection with decapitation experiments. Söding⁽⁴⁰⁾ has drawn attention to the great loss of sap from decapitated stumps, and many authors have emphasised the difficulty thus put in the way of recementing the tip in position (p. 230). It is a frequent comment (e.g. Jensen⁽⁷⁾, Purdy⁽²⁸⁾) that for concordant results the humidity at which the seedlings are kept must not vary beyond certain limits, i.e. guttation may occur too freely or not enough.

Söding points out also that bleeding may occur very irregularly from the cut stump, but is more regular after a drop of gelatin is placed on the cut end. This is probably to be explained as follows. The sap wells up from the vascular tissue and whether it exudes in regular drops or not depends upon its power of wetting the rest of

the exposed surface, and this will depend upon the nature of the surrounding walls, and their degree of wetness, etc. If, however, the whole surface is covered with moist gelatin, the drop will form over the whole cut end before it falls, and the drops should appear in regular succession and of about equivalent size. Söding finds further that if the apex is replaced on this stump by gelatin, the growth rate of the stumps, though by no means equivalent to that of the uninjured coleoptile, is greater for the next five hours than that of the untreated stump. On the other hand after 13 hours the stump alone is making better progress than stump plus replaced tip.

Söding explains these facts in this manner. Growth was accelerated normally by hormones formed in the tip, when the tip was removed the hormones were lost and the growth rate diminished; after 13 hours, the tip is regenerating in the untouched stump and the hormones are reappearing; he adds in support of this view that if the regenerating tip is again removed the growth rate again falls. When the original tip is replaced by gelatin, Söding suggests that the hormones in the tip diffused through partially and thus accounted for the greater growth in the first five hours, but as the presence of the replaced tip prevented the regeneration of a new apex at the cut surface of the stump, at the end of 13 hours this stump with replaced tip is falling behind in growth rate.

A study of wound healing (26, 27) supplies no instance of the regeneration of a new tissue in the short space of time of 13 hours, particularly in a tissue which is completely vacuolated, and Söding brings absolutely no evidence in support of his unlikely hypothesis. The point admits of direct experimental investigation¹, and until such evidence is forthcoming this suggestion may be disregarded, particularly as there is a much simpler explanation.

When the tip is removed the stump bleeds for a time and then blocks naturally as a result of drying and oxidation processes at the cut surface (27). When the tip is put back with gelatin a partial block is produced immediately, less loss of sap occurs and the fall in growth rate is less marked during the first five hours. During the next few hours, however, loss of sap continues through the moist gelatin, where the replaced tip prevents much oxidation. The exposed stump, on the other hand, blocks completely; the sap is now no longer lost and extension growth is now stronger in the stump without replaced tip.

¹ It has been investigated by Miss Tetley in this department, with the result that Söding's suggestion has been found to have no experimental basis.

When the tip is replaced, the block would probably be more effective if the apex were replaced without gelatin. This is perhaps the meaning of Paál's experiment with *Coix* (p. 232), the stump on the side blocked by the apex is alone able to accumulate sap, but when gelatin is added the whole surface continues to bleed and so no curvature results.

When Snow (39) inserted an apex laterally upon a stump, apart from the lateral illumination effects, if both apex and stump were still capable of growth, greater growth was perhaps to be expected on opposite sides in the two pieces, namely—on those sides which were partially blocked. This result is the one which Snow obtained. Snow's experiments were carried out so far as possible under conditions which avoided excessive guttation so that in spite of the gelatin local blocking may have been obtained.

The effect of gelatin in thus maintaining bleeding from the cut surface will largely be prevented by the backing of an *impermeable* cap of tissue, and in this sense Stark's experiments may be interpreted.

The effect of a foreign apex will then be determined by its degree of "fit," and obviously there is likely to be a closer fit between the severed pieces of closely allied species, but closest fit when a tip is replaced on its own stump. When, however, a light is thrown laterally upon the tip, the walls of the cells on one side become markedly more permeable, gelatin and sap will then ooze from the cut surface into the tissue of the apex on this side, and the vein on this side of the coleoptile will lose more sap than the other, and as a result basal extension of the coleoptile will be less on this side than the other. From this standpoint it is clearly immaterial whether the unilateral lighting is applied to the apex before or after it is replaced on the stump, the effect will be the same. On the other hand, Brauner's experiment in which a completely etiolated tip was replaced on a laterally illuminated stump is probably to be explained either through the washing of gelatin and sap away from the cut surface via the stump more freely upon the illuminated side, or more probably to the shorter length of the extended cells in the stump as a direct result of illumination.

Many of the data in relation to decapitation will require still further analysis. At first sight, for instance, a recent result of Boysen-Jensen and Nielsen (7) is inexplicable (Text-fig. 4). When two apices were placed upon one cut stump and laterally illuminated, one apex being protected by platinum foil from the light, then a *negative*

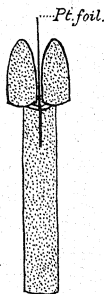
phototropic curvature occurs in the stump. Under illumination the apex towards the light should presumably become more permeable and thus a greater leak take place on this side. These stumps are placed on, however, without gelatin, and the authors observe that only within a certain range of humidity conditions is this experiment successful, water must not guttate freely nor must the stump become too dry. Within the finely balanced range of exudation pressures, it is obvious that the slight drying of the illuminated side in the light may just favour efficient blocking at the top of the cut stump and negative curvature result.

The different results of placing laterally on the stump, an apex or a ring of tissue from the coleoptile as reported by Nielsen (p. 233) now seem quite explicable. The ring of tissue, unless cut from very near the apex, has two veins running completely through it and when placed on the side of the stump fails to block the flow of sap and at the same time prevents the natural blocking on this side; the result is, beneath the ring more sap is lost and curvature of the stump is towards the side bearing it. The apex, on the other hand, blocks the stump on the side on which it is inserted and the stump on this side loses less sap and bends away from this flank.

Before leaving these phenomena of guttation it is necessary to point out that the steady flow of sap upward through the coleoptile is very difficult to reconcile with the assumption that growth-regulating substances or hormones are diffusing *from apex to base*. These substances, presumably diffusing in water, are thus assumed to be diffusing against the stream.

As the flow of sap from the tip of the coleoptile is always vigorous and is favoured by the incidence of light upon the tip it is not easy to see how the downward distribution of the growth-regulating substance could be effected.

Another series of experiments, mentioned in the previous section, in which is examined the effect of cutting a vein upon movements beneath the cut, now require brief consideration. In Boysen-Jensen's experiments, when a vein is cut on the side away from the light, the additional loss of sap from the cut appears to balance the loss of sap on the flank as the result of illumination, and no tropic curvature results. It is difficult to see, however, how it is that the curvatures still persist when both veins are severed in saturated air. The problem



Text-fig. 4

is probably on all fours with that provided by Paál's experiments in which the hollow coleoptile alone is employed, with its base inserted in moist sand and the organ in a saturated atmosphere. Under these conditions, however, there is a long series of experiments to show that the living tissues can develop demonstrable sap pressures. The sap pressures recorded by Kraus for isolated pieces of plant inserted in moist sand (18) were slight, but they are probably sufficient to account for the slight growth and slight curvatures of the coleoptile which still take place, and when these slight exudation pressures are still further diminished by increased permeability of the replaced apex on one side as the result of lateral illumination, the difference in sap supply to the two sides may be very significant in connection with the small amount of length extension now possible to the organ.

It is contended that from this new standpoint all the recorded phenomena fall simply into line. The difficult problem as to the succession of positive and negative phototropic curvatures in lateral illumination of a certain strength and duration remains to be elucidated, as also the fluctuation of growth rate with which these curvatures are possibly correlated.

In this connection, however, it is easy to see that there are a number of contending factors. Under continued illumination, a rapid growth rate on the more brightly lit side will be followed as the process on this side nears its end by a more rapid rate upon the shaded side, so that so far as this cell extension effect is concerned, negative and positive curvatures may succeed one another. On the other hand, apical guttation may be greater first on the illuminated side, with positive response, followed by a greater loss from the other side as the light effect continues to develop, and a second negative curvature. Such effects as these superimposed may obviously give a series of fluctuations in growth rate and tropic curvature which are difficult to analyse, but at least it is rational to find that *with the apex shaded*, the curvatures in strong light are less complicated and the correlation between extension and curvature more plainly seen.

It must not be forgotten, further, that with strong light in a normal atmosphere, instead of guttation controlling extension and curvature the rate of evaporation may become the significant factor, and in this case the side towards the light may lose most water with another form of positive phototropic curvature as a result (24). From this new standpoint, the facts now take their place amongst a wider

range of experimental and observational data relating to this same organ. Furthermore this interpretation of the phototropisms of the coleoptile brings these phenomena into line with the "light-growth" theory which Blaauw has used so successfully in the explanation of phototropic curvature in the normal etiolated shoot (2, 3, 4).

Had it not been for the difficulty of the apparent transmission of a stimulus to a distant responsive region in the coleoptile this theory would probably already have won universal acceptance.

It is surely, therefore, very significant that an attempt to interpret the behaviour of the coleoptile which is in harmony with the light-growth theory of Blaauw, appears to meet with fewer difficulties and involve fewer inconsistencies than any based upon the classical theory of stimulus, transmission and response.

SUMMARY

1. Recent experiments with the coleoptile in which the apex is replaced upon the decapitated stump are supposed to demonstrate that lateral light falling upon the apex causes the production or release of growth-regulating substances which diffuse to the lower part of the coleoptile and there cause phototropic curvatures.

2. Such an experimental demonstration of the reception of a tropic stimulus in one region, followed by tropic response in another, if fully established, excludes the possibility of explaining phototropic curvature as a simple "light-growth" reaction such as is suggested by Blaauw.

3. But when the numerous facts as to the conduction of phototropic curvature from the decapitated apex are considered it is found that their explanation in terms of the classic theory of stimulus and response entails numerous difficulties, and some of the assumptions made at present are mutually inconsistent. The facts are therefore reconsidered after the structure and growth of the coleoptile has received fuller elucidation.

4. This organ possesses limited growth and during the period it is employed in phototropic experiments its increase in length is entirely by cell extension; no new cells are formed.

5. This process of cell extension proceeds more rapidly in light, but the cells do not stretch so much; in the light therefore the coleoptile grows more rapidly but its final length is less.

6. The frequent occurrence of apical guttation is recalled, it is shown that the localisation of the apical hydathode explains the direction of the usual autonomous curvature of the coleoptile.

7. The permeability of the coleoptile tissues are increased by light, and therefore light falling on the apex increases the apical guttation.

8. Lateral light falling on the apex therefore increases the rate of flow from the vein nearer the light and thus produces a phototropic curvature.

9. When the apex is removed, guttation is very free and growth much reduced until the cut surface is blocked.

10. If the apex is replaced immediately, the guttation is reduced and growth is greater. If the apex is replaced on the side of the stump only, this side only is blocked and curvature results.

11. From the same standpoint the different curvatures produced by the asymmetric replacement of an apex or a ring of coleoptile tissue are explicable.

12. If the replaced apex is laterally illuminated the block is less complete on this side of the stump and positive tropic curvature results.

13. The various facts at present elucidated by investigators as due to growth regulation by substances diffusing from the apex, are passed in review from this new standpoint, and appear to be consistent with it.

14. On the other hand the fact of the upward flow of guttation, reported by nearly all experimenters with decapitated coleoptiles on which tips are replaced, is not easily reconciled with the idea of the downward diffusion of growth-regulating substances.

15. It is then shown that an interpretation of the phenomena of phototropic curvature in the coleoptile which is consistent with the "light-growth" hypothesis of Blaauw can be applied with less difficulties and fewer inconsistencies than any interpretation yet attempted on the lines of stimulus and response.

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MYCORRHIZA

By M. C. RAYNER

CHAPTER VII

Recent researches on endotrophic mycorrhiza other than, that in Orchidaceae and Ericaceae—Peyronel's theory of "double infection"—Jones—Demeter—McLennan; fungus infection in *Lolium*—Constantin—Fossil Mycorrhiza: Weiss; Osborn.

THE accounts of many independent observers will have made clear to the reader that the fungi concerned in the formation of endotrophic mycorrhiza fall into two fairly distinct groups in respect to their morphological characters. In one group are the Orchid endophytes, the mycelium of which possesses numerous cross walls and develops characteristic skeins or "pelotons" in the mycorrhiza cells. In pure culture these fungi form characteristic conidia and, whatever their immediate systematic affinities, they clearly belong to the Higher Fungi. In another group may be placed the endophytes of a majority of the herbaceous flowering plants, excluding Ericaceae and certain other obviously specialised groups. These are characterised by the formation of special organs—the "vésicules," "arbuscules," and "sporangioles" recorded and figured by many observers. Vesicles have been variously interpreted as reproductive organs or as vegetative structures functioning for the storage of reserves; arbuscules are generally regarded as of the nature of haustorial branch systems, recalling those formed by members of the Peronosporaceae; as a result of digestive activity on the part of the host cells they assume an appearance formerly interpreted as due to the existence of special organs—the "sporangioles" of Janse and other workers.

The widespread distribution of intracellular infection of the latter type was first put on record by Gallaud, who described and illustrated mycelium bearing arbuscules and vesicles from the roots of a large number of species of very varied affinities.

The presence of mycelium belonging to two distinct types in the mycorrhiza of flowering plants was suggested by the records of more than one observer and had led to some confusion in the literature, but the actual occurrence of such a condition was purely conjectural.

Of observations pointing to such a composite type of infection may be mentioned those by Mollberg (1884) on *Epipactis* and *Platanthera* describing "vésicules" in the roots of those Orchids in addition to the characteristic intracellular "pelotons," and also those of Gallaud (1915) on the presence of "pelotons" resembling those of the Orchid fungi in the root cells of *Tamus* and *Psilotum*, side by side with typical arbuscules. Gallaud noted also that the roots of certain plants, e.g. *Allium sphaerocephalum* and *Ruscus aculeatus*, contained mycelium bearing arbuscules and vesicles with which were associated stromata similar to those produced by the Orchid fungi in pure culture. These and similar inconsistencies of structure were believed to depend upon the reaction of the endophyte to physiological differences in the root cells.

The observations recorded by Petri (1918-1919) on Vine and Olive were even more confusing. By placing mycorrhizas of these plants in moist chambers and allowing them to decay, this worker believed that he had secured evidence that the same mycelium which bore vesicles and arbuscules within the mycorrhiza cells, produced on the surface conidial fructifications quite analogous to those observed in cultures of the Orchid fungi by Bernard and Burgeff. Finding, as he believed, the same type of mycelium in plants so diverse as Vine, Olive and the Orchids, Petri deduced the conclusion that the fungi of endophytic mycorrhiza belonged to a single group, thus endorsing the views expressed earlier by Janse and Gallaud.

The theory involving explicit recognition of simultaneous infection by two distinct fungi is due to the Italian botanist Peyronel. The work of this observer on the fungi concerned in the formation of tree mycorrhizas will be discussed in a later section of the present work (Peyronel, 1920, 1921, 1922, 1924). In addition, he has recently reviewed and criticised the views expressed by other observers on the aspect of endotrophic infection now under consideration, and has carried out independent observations on the same subject. His own researches were designed to test the hypothesis of dual infection in endotrophic mycorrhizas showing, as he believed, two distinct types of mycelium. His observations have led to the publication of a new theory of endotrophic infection to which attention must now be directed (Peyronel, 1922, 1923, 1924).

From the time of his earlier researches on Wheat, Peyronel was struck by the dimorphic character of the mycelium in the mycorrhiza of that plant, the differences being sufficiently well marked, in his view, to suggest the presence of two independent fungus species.

After long consideration he rejected this view, partly by reason of the arguments put forward by other workers, and partly because the two types of mycelium were not infrequently present in the same cells and did not lack structural features in common. His later observations, however, have led to a different interpretation of the facts, in sharp contrast with the opinions of Janse, Gallaud and Petri, and with his own earlier views. Peyronel's conclusions may be summarised briefly as follows.

The endophytes of Orchids on the one hand, and of the majority of flowering plants on the other hand, belong to two entirely different groups of the Fungi; the former are members of the Eumycetes, the latter show characters resembling those of the Phycomycetes. In each case, a number of biologic forms belonging to one or a small number of species are probably included. It is regarded as certain by Peyronel that endotrophic infection in a majority of mycorrhiza plants is of composite character, involving the presence of two endophytes; one, usually less developed, of the *Rhizoctonia* type familiar in Orchids, the other showing Phycomycete characters. The mycelium of the latter is recorded as widely distributed in damp humus soils, where it forms a fine network investing the roots of plants and passing from one to another. It flourishes under purely saprophytic conditions and may be found growing strongly in dead tissues *in situ* and in fragments of plant material in the soil. When living saprophytically it produces vesicles and lateral branch systems analogous to arbuscules.

In a majority of plants with endotrophic mycorrhiza, infection by the Orchid type of fungus supervenes upon that by the mycelium producing vesicles and arbuscules. The former develops chiefly in the outer cortex where it overgrows that producing arbuscules; it behaves rather as a *quasi*-parasite or saprophyte than as a true symbiont and is easily extracted and grown on artificial media.

In many endotrophic mycorrhizas, therefore, two distinct phases of infection may be recognised; an earlier, depending upon invasion by a fungus of the familiar arbuscule-producing type, and a later, in which a second organism of the *Rhizoctonia* type is also present. Fungi showing characters similar to the *Rhizoctoniae* of Orchids have been isolated by Peyronel from a number of different species of flowering plants, including several cereals, viz. *Triticum sativum*, *Zea mais*, *Hordeum vulgare*, *Solanum tuberosum*, *Nicotiana tabacum*, *Daucus carota*, *Beta vulgaris*, *Arum italicum*, *Euphorbia peplus*, *Circaea alpina* and *Saxifraga rotundifolia*. All the endophytes resembled one another and behaved in pure culture like those isolated from Orchids by

Bernard, Burgeff, and Constantin and Dufond. Peyronel has accordingly accepted the generic name *Rhizoctonia* for these fungi. The form isolated from Wheat has been re-inoculated into aseptically seedlings and has reproduced a mycorrhiza with typical "pelotons" but without the arbuscules and vesicles ordinarily also present in Wheat mycorrhiza (Fig. 46).

The endophytes belonging to the other group have not yet been isolated, but Peyronel has observed mycelium in dead roots and in soil similar to that in living roots. He has also identified it in cork and in fragments of bark of pot plants of *Citrus*, *Olea europaeus*, and *Morus nigra*. Moreover, when dead roots known to be infected were examined at intervals during the autumn and winter, the production of

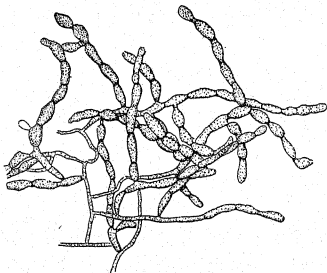


Fig. 46. Mycelium and moniloid chains produced in pure culture by a form of *Rhizoctonia* isolated from Wheat. Compare fig. 15 showing similar structure of an Orchid endophyte. Original $\times 375$. Reduced about $\frac{1}{2}$. (From Peyronel, 1924.)

large numbers of vesicles could be observed. Peyronel's recent researches on dead roots of Wheat, Maize and wild grasses are reported to have established the direct relation of the vesicles with mycelium bearing arbuscules, so confirming his view that the former are sporangia of the same fungus in various stages of development. The spores eventually formed are constant in size and shape and are set free by changes in the sporangium wall (Fig. 47).

In the absence of sexual organs the evidence at present available is not regarded as sufficient to assign these fungal forms to a definite systematic position. It is suggested that they show affinities with the genus *Endogone*, a small group classed at one time with the Gasteromycetes or the Tuberaceae, more recently assigned to the

Hemiascii by Baccarini (1903) and to the Oömycetes by Bucholtz (1911). The sporangia of *Endogone macrocarpa* figured by Tulasne and reproduced by Schröter and Fischer are noted by Peyronel as remarkably like the vesicles produced by many mycorrhizal fungi.

Peyronel has likewise drawn attention to the isolation by Bernard (1909, 1911) of a fungus showing Phycomycete characters from the roots of *Solanum dulcamara*. A similar form was subsequently extracted from plants of the same species by Magrou (1921) who named

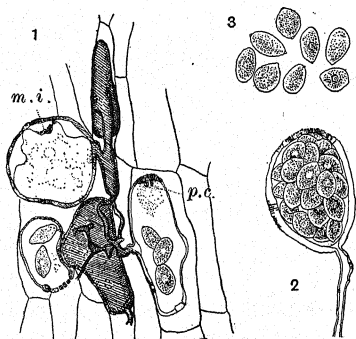


Fig. 47. Fructifications of an endophytic fungus bearing vesicles and arbuscules, from Maize and Wheat. 1. Group of ripe sporangia: *m.i.* internal membrane of a sporangium partly detached from the external membrane; *p.c.* conical processes on the internal surface of the sporangium wall. 2. Ripe sporangium with spores. 3. Spores. Original $\times 500$. Reduced about $\frac{1}{2}$. (From Peyronel, 1923.)

it *Mucor solani*. He has pointed out also that the existence of what may be called a *normal radicolous flora*—"flora radicola normale"—on the roots of wild and cultivated plants at a certain stage of development is of practical importance in view of the fact that many of the fungi present are facultative parasites, the excessive development and virulence of certain constituents being possibly directly related to soil conditions. It is believed that the development of mycorrhiza in general is greatly accentuated in soils seldom cultivated and permeated with roots; hence its abundance in pasture fields and relative infrequency in cultivated plants and in ruderals.

It has recently been stated by Magrou (1921) that annual plants do not form mycorrhiza, or do so in a very transitory form. This statement is not acceptable to Peyronel, who has observed vigorous and persistent mycorrhizas in many annual plants under suitable conditions, e.g. in *Triticum aestivum*, *Hordeum vulgare*, *Zea mais* and *Secale cereale*. The theory of Bernard (1909) associating the perennial habit in general with fungus infection has also been rejected as incompatible with his own observations.

Peyronel (1924) has recently published a long list of species in the mycorrhiza of which he had observed the dual type of endotrophic infection. They are distributed among 37 families, so that, assuming the correctness of these observations, this type of infection may be regarded as widespread among flowering plants.

It is of interest to compare Peyronel's observations on leguminous species in Italy with those recorded independently by Jones (1924) in the mycorrhiza of a number of cultivated legumes in the United States. Jones' observations were made first upon peas and subsequently extended to other crop plants. He noticed that a majority of apparently healthy lateral roots of the former plant showed conspicuous discoloration due to infection by a coarse non-septate mycelium with a profuse development of arbuscules and vesicles, i.e. of the characteristic Phycomycete type distinguished by Peyronel. Infection of this kind was observed also in greater or less degree in the roots of other leguminous crop plants, four of which, *Melilotus officinalis*, *Medicago sativa*, *Trifolium pratense* and *T. repens* are included in Peyronel's list (published subsequently) as showing dual infection. Neither in the account given by Jones nor in his figures is there any suggestion of the presence of another endophyte with distinct morphological characters. Since the *Rhizoctonia* type of infection follows the other, this omission may have been due to the age of the roots, or the distinction of the two types of mycelium may have been overlooked by the American observer. Attempts to extract the fungus from infected roots were unsuccessful.

The observations made by Jones are obviously incomplete, but are of interest as indicating the uniformity and widespread character of the Phycomycete type of mycorrhizal infection. While admitting that the relations of the root cells with the mycelium were not of the ordinary parasitic kind, he was not able to detect any beneficial effect upon the host plants, and indeed observed indications that leguminous crop plants benefited when protected from root infection by sterilisation of the soil before planting. Decisive evidence bearing

on this matter can be obtained only by isolation of the endophyte or endophytes and "pure culture" experiments with infected and uninfected plants, and even then the results must be applied with great caution to plants growing naturally.

Under the name of "*Plasmoptysen-Mykorrhiza*" Demeter (1923) has recently described a type of root infection characteristic of Asclepiadaceae and Apocynaceae, with special reference to the mycorrhiza of *Vinca minor*. On experimental grounds he concluded that the mycotrophic habit is obligate in *Vinca* when growing under natural conditions, about two-thirds of the roots showing infestation. The manner of infection is related by the author to the presence of special cells, "Kurzzellen," in the sub-epidermal layer (exodermis) of the root, corresponding to the "cellules de passage" mentioned by Janse (1897) and the "Durchlasszellen" of Burgeff (1913) in other plants. Root infection is continuous throughout the year from early spring onwards. The general type of structure is that made familiar by Gallaud (1905): the mycelium, mainly intercellular in distribution at first, becomes intracellular owing to the development of haustorial branch systems or "arbuscules" which undergo degeneration later to structureless sporangioles. Terminal and intercalary vesicles of the usual kind are freely produced.

The specific name given to this mycorrhiza by the author followed upon a cytological study of the arbuscules, of which the tips of the ultimate branchlets lose their identity, and become imbedded in a finely granular matrix staining deeply with haematoxylin. This structure is attributed by the author to bursting of the hyphal tips and liberation of the contents owing to the action of acid present in the cell sap. Alternatively, it is suggested that it may be brought about by an excessive rise in the osmotic content of the hyphae, due to enzyme activity and the intake of sugar (see also Burgeff, 1913). Demeter favours the former interpretation and has obtained evidence of similar behaviour on the part of the mycelium in artificial cultures when the fungus is grown on media containing hydrochloric acid at a concentration of about 0.025 *N*. The structures described were named "Plasmoptyse" by the author, who has accordingly adopted the name "Plasmoptysen-Mykorrhiza" for the type of mycorrhiza found in *Vinca minor*. Careful study of the figures supplied leaves some doubt as to how "Plasmoptyse" differ from the earliest phase in intracellular digestion, i.e. the initial stage in the conversion of arbuscules into sporangioles. In respect to the identity of the endophyte, the conclusions reached also provoke criticism. Fungal

forms showing identical characters were isolated independently from plants growing in three separate localities. Under cultivation, this fungus, believed by Demeter to be the endophyte, showed morphological characters resembling those of the Orchid fungi and was named *Rhizoctonia apocynacearum* accordingly. On the other hand, it is recorded that marked dimorphism of the hyphae and great variability were exhibited in single cultures, according to the nature of the nutrient supplied. The author figures mycelium and conidia resembling those of the Orchid endophytes from artificial cultures and alludes also to the production of "arbuscules" and "Plasmoptyse" in similar colonies, "Diese 'Plasmoptyse' wurde an den in Reinkultur gezogenen Endophyten kunstlich ausgeführt und erfolgte im Optimum bei einer Säurekonzentration von etwa 0.025 N HCl..." but he does not figure mycelium bearing these structures and also conidia simultaneously. In view of Peyronel's (1924) recent conclusions respecting dual infection, i.e. the existence in many plants of secondary infection by a fungus of the *Rhizoctonia* type superimposed upon primary infection by an endophyte bearing vesicles and arbuscules, Demeter's views challenge attention. The production of conidia of the *Rhizoctonia* type in pure culture by a mycelium which also produces vesicles and arbuscules has never before been recorded and is in direct disagreement with Peyronel's conclusions. Indeed, the isolation and cultivation of an endophyte producing arbuscules is recorded for the first time in this paper. Inoculation experiments did not reproduce the mycorrhizal condition observed in roots growing naturally.

Peyronel (1924) has since included *Vinca minor* in his list of plants showing the dual type of infection. In view of this, and of the unsatisfactory character of the evidence supplied by Demeter in respect to the identity of the endophyte isolated by him, the conclusions reached by the latter require confirmation.

The claim put forward by Peyronel that "double infection" is a frequent and regular phenomenon in endotrophic mycorrhiza is of considerable interest as providing a reasonable explanation of certain observed inconsistencies of structure. It is supported by evidence of a suggestive kind but can hardly be regarded as definitely established. In the more specialised groups, evidence for its occurrence is at present almost negligible; records are extremely rare for Orchids and do not exist for members of Ericaceae. It may be that greater specialisation to the symbiotic relation carries with it an increased resistance to invasion by the "Phycomycete type" of mycelium. On the other hand, the fact that the latter has hitherto resisted iso-

lation, except in the not very satisfactory case reported by Demeter, in itself constitutes evidence of relatively great specialisation in respect to the symbiotic habit, and is somewhat inconsistent with the wide saprophytic distribution reported in dead roots and organic soils. The endophytes of the *Rhizoctonia* group are described as showing morphological characters identical with those of the Orchid fungi. The latter show a high degree of specialisation in relation to particular hosts, although they grow as saprophytes with comparative readiness in pure culture. Peyronel himself has alluded to the *Rhizoctonia* forms from "double mycorrhizas" as behaving rather as saprophytes than as true symbionts, and he isolated them with comparative ease from a number of mycorrhizas. It is possible that edaphic factors may be of importance and it is evident that the whole subject offers a profitable field for further investigation.

The endophytic fungus of *Lolium* described by McLennan (1920) merits notice, more especially in view of the occurrence of seed-coat infection in Ericaceae. The existence of fungal infection in the grains of Darnel Grass (*Lolium temulentum*) had long been known. Papers dealing with the subject were published by Vogl (1898), Guérin (1898), Hanausek (1898) and Nestler (1898). Hiltner (1899), working at the physiology of the relation, believed that a case for nitrogen fixation had been experimentally established. Subsequently papers appeared by Micheletti (1901) and Freeman (1903), the latter recording sporadic infection of a similar kind in grains of other species of *Lolium*.

The whole subject has recently been reinvestigated by McLennan (1920), who has shown that the occurrence of fungal infection in the genus *Lolium* is wider and more constant than was believed. Infection of the grains of *Lolium temulentum* and *Lolium perenne* was found to be the rule, from whatever part of the world seed was obtained. The ultimate origin of infection in the genus is still unknown. Mycelium is present in the embryo-sac at, or immediately after, fertilisation, before the ovum divides. Infection spreads into the nucellus and carpel walls, but is limited in extent by the absorption of mycelium, which is apparently utilised as a source of food supply by the developing embryo. Ordinarily, a persistent layer is formed around the endosperm, although—"if the fungus does not keep pace with the absorbing power of the endosperm, no hyphal layer is formed in the ripe grain, but hyphae can then be found in the scutellum and embryo." After germination the mycelium grows *pari passu* with the seedling tissues, following the development of the stem apex.

The distribution of the mycelium is mainly intracellular and individual parenchyma cells of the young grass stems may contain a dense network of hyphae. At the flowering period mycelium is abundant at the base of the carpels, whence hyphae extend to the developing ovules. All attempts to isolate the fungus have been unsuccessful and no experimental evidence was obtained by McLennan in support of the view that nitrogen-fixation is a function of the endophyte. Nevertheless, it is believed that—"the association of the fungus with *Lolium temulentum* and *Lolium perenne* is probably a well-marked case of symbiosis, comparable in many respects with that met with in *Calluna vulgaris*." The formation of (root) mycorrhiza in *Lolium* had not been recorded by any observer at the time this paper was written. McLennan (1926) has since made a further contribution to the subject, recording the presence of endotrophic mycorrhiza in the roots of *Lolium* and describing in detail the cytology of root infection in one species, *L. temulentum*.

For some unexplained reason the formation of mycorrhiza by members of Gramineae was long overlooked. Schlicht (1899) noted it in *Holcus mollis* and *Festuca ovina*: Schröter (1908) recorded his own observations and those of Schnellenberg on various alpine grasses. Jefferies (1916), describing the piliferous layer of the root of the Purple Heath Grass (*Molinia coerulea*), noted the frequent presence of mycorrhiza—"the mycelium being visible in the cells and on their surface, and occasionally penetrating to the cells of the second row." More recently, Peyronel (1922) described the mycorrhiza of Wheat and a number of other cereals as due to infection by a fungus producing the characteristic arbuscules and vesicles associated with endotrophic infection. To these records is now added that of McLennan for four species of *Lolium*, namely, *L. temulentum*, *L. perenne*, *L. multiflorus*, and *L. subulatum*.

The mycorrhizal fungus of Darnel Grass is of the Phycomycete type distinguished by Peyronel, characterised by the production of vesicles and arbuscules and the conversion of the latter organs to sporangioles as a consequence of digestive activity. The distribution of mycelium is similar to that recorded for *Arum maculatum* by Gallaud, the hyphae, intracellular in the outer layer of the root, becoming both inter- and intra-cellular in the deeper tissues. The mycorrhiza is described as "a particularly favourable one for studying the cytological details," and McLennan has drawn certain inferences respecting exchange of nutritive material from an intensive study of the infected cells. These will be considered later when reviewing the

evidence relating to nutrition in mycorrhiza-plants in general. (Figs. 61, 62.)

There is at present no evidence whatever bearing on the identity or otherwise of the two endophytes of *Lolium*, i.e. the mycorrhizal fungus proper and that associated with the fruit, seed and vegetative tissues of the shoot. The distribution of mycelium in the tissues of the seedling inevitably challenges comparison with that of various members of the Ustilaginaceae. It is greatly to be desired that future work may render it possible to isolate and cultivate the fungus or the fungi present in *Lolium*, and thus throw light on the systematic affinities of the endophytes and provide further evidence as to the biologic relations existing in this interesting case of symbiosis.

Another remarkable case of endophytic fungus infection other than that responsible for mycorrhiza formation has been reported in the family Bromeliaceae. On the older view, the epiphytic members of this group were reported to obtain the requisite nutritive materials from the decomposition products of vegetable remains and small insects caught in the gummy secretion of the leaves. Certain recent observations on *Tillandsia dianthoides* provide evidence that this, at least, is not the only explanation. A plant of this species, hanging freely from a wire, was kept under observation for fifteen years and observed to grow and flower regularly; no evidence was found that any mechanism existed for holding water or for capturing and digesting insects. Like other epiphytic bromeliads, this species of *Tillandsia* forms scale-like structures or "lepidotes" upon the surface of the shoot. On investigation, these proved to be small receptacles connected with deep-lying groups of cells. The cavities, in addition to the presence of organic and inorganic detritus, showed regular infection by a septate mycelium present in relatively great abundance. The fungus responsible was isolated and identified as a species of *Volutella*. It is assumed that the relation between the *Tillandsia* plant and the fungus is one of beneficial symbiosis, the latter supplying nitrogenous food material to the green plant (Dubois, 1925 a, b, c).

Chiovenda (1918) has recorded the rare fungus *Myriostoma califorme* for the second time in Italy. It was found closely associated with the roots of *Polygonum persicaria*, in which it was believed to be responsible for the formation of endotrophic mycorrhiza.

Ramsbottom (1922) has drawn attention to the association of small undifferentiated seeds with obligate mycotrophy in the families Orchidaceae, Burmanniaceae, Ericaceae, Pyrolaceae, and Gentian-

aceae, and has noted the numerous records of difficult or unsatisfactory germination and their possible significance in relation to fungus infection. The mycorrhizal associations existing in Gentianaceae have been insufficiently explored and would probably repay experimental study. Many of the members of this group are plants showing marked edaphic peculiarities, and not a few of them are difficult subjects in horticultural practice. Frank (1887) and Schlicht (1889) cited *Menyanthes trifoliata* as a typical case of non-infection, and the latter even mentions Gentianaceae as a group in which he had been unable to find infection in the plants selected for examination. "Von den Familien aus denen ich Pflanzen untersucht habe, konnte ich eine Verpilzung der Wurzeln nicht beobachten bei folgenden: Crassulaceae, Scleranthaceae...Gentianeae...Cariceae."¹ Stahl (1900) alluded to the existence within the group of species recorded as free from infection, e.g. *Limnanthemum* and *Menyanthes*; together with the holosaprophytic species of *Voyria* described by Johow (see Pl. I). Stahl himself provided a long list of species—alpine and other species of Gentian, *Erythraea centaurium* and *Chlora perfoliata*, in which he had observed regular infection. Schröter (1908) afterwards confirmed these observations for a number of alpine Gentians. It was suggested by Holm (1897) that the curious gentianaceous species *Oblaria virginica* was a connecting link between the typical green members of the family and the saprophytic forms *Voyria* and *Voyriella*. In *Oblaria* the green colour of the shoot is masked by the presence of anthrocyanin in the epidermis; the root system shows a "coralloid" habit and typical endotrophic infection; there are no root hairs.

The relation of mycorrhiza to pathological conditions in roots has interested mycologists from the time of Theodor Hartig onwards. Discussing this matter in reference to certain plant diseases, especially those of Sugar Cane, it has been pointed out recently that disturbance of the normal activity of the mycorrhizal fungus may lead to secondary invasion of roots by bacteria or parasitic fungi, e.g. species of *Marasmius* or *Rhizoctonia* (Constantin, 1924). Parasitic members of the latter genus cause much damage to plants and some of the pathogenic forms closely resemble the endophytes of Orchids (Constantin, 1925). Observations of this kind raise questions of great practical interest, especially in relation to tropical agriculture. It may be possible to correlate them with isolated observations by other

¹ The only member of Gentianaceae mentioned in Schlicht's list of species examined is *Menyanthes trifoliata*!

workers, e.g. those on the bacterial investment of ericaceous roots in calcareous soil (Rayner, 1913), and also with the formation of "Pseudomykorrhiza" in the Conifers, and the invasion of roots of these trees by fungi of a more or less pathogenic character under certain soil conditions (Melin, 1923, 1925).

In view of the possible affinities of the family Aristolochiaceae with parasitic or semi-parasitic groups like the Rafflesiaceae, Loranthaceae and Santalaceae, the formation of mycorrhiza by *Asarum europaeum*, recorded by Schwartz (1912), is noteworthy.

In this plant, an uncommon and somewhat doubtful member of the British flora, the young adventitious roots are reported to form endotrophic mycorrhiza of a typical kind. It is apparently inconstant in appearance, being absent from individual plants from certain stations and also from *Aristolochia sipho*, another member of the same group. Schwartz described and figured intracellular infection of the inner layers of the cortex, with disappearance of starch from infected cells. It was noted that the endophyte resembled those of *Thismia* and *Neottia*. In the outer cells of the infected zone the hyphae formed rather loose coils or "pelotons" while the cells of the innermost cortical layer showed typical intracellular digestion of the mycelium. The formation of "arbuscules" was not observed, but the author recorded the presence of spherical and pear-shaped vesicles of the familiar type. In the light of Peyronel's "double infection" hypothesis and in view of Schwartz's tentative suggestion that in *Asarum* the mycelium bearing these may belong to a different fungus, the mycorrhizal condition in *Asarum* might repay further investigation.

The part played in nutrition by the fungi present in endotrophic mycorrhiza, and the biological significance of root infection in relation to differences of soil and other external factors continues to be a subject of controversy. There is a strong *prima facie* case for a beneficent action upon the nutrition of the host based upon cytological and microchemical evidence. To supplement this, data derived from pure culture experimental researches on certain groups is now slowly accumulating. The whole subject will be dealt with more fully in a later chapter.

Fossil Mycorrhiza

In view of the wide distribution of mycorrhiza at the present day and also of its biological significance, observations suggesting its occurrence in fossil plants are of considerable interest. The evidence relating to fossil fungi in general was summarised by Seward (1898),

who concluded that there is fairly good evidence for the existence of phycomycetous fungi in Permo-carboniferous times, the earliest reliable records for the Higher Fungi occurring much later, in Post-paleozoic or even Tertiary times.

The "coal-balls" from which so much of the evidence relating to Paleozoic plants has been derived, often contain mycelium in and about the fragments of roots and other plant organs which they enclose. Records of hyphae in the tissues of plant fossils are also common, and the mycelium occasionally shows characteristic morphological features, e.g. the presence of spherical or pear-shaped swellings or vesicles. Mycelium identical in appearance is associated also with plant fragments in peat deposits of various ages and belongs almost undoubtedly to fungi growing saprophytically in a matrix of decomposing plant residues. Some of the fossil records are believed to point also to the occurrence of parasitic mycelium in plant tissues, and in general, the evidence relating to fossil fungi indicates a mode of life similar to that of the forms now in existence.

In the cortical tissues of *Lepidodendron* or *Stigmaria*, Seward has figured mycelium distributed in the middle lamellae of the cell-walls and forming localised swellings or vesicles within the cells. This recalls the habit observed in endotrophic mycorrhizas at the present day but the material is too scanty to constitute a definite claim for the existence of mycorrhiza in members of this group of fossils. There are, however, at least two records pointing to the formation of mycorrhiza by Paleozoic plants.

Weiss (1903) described a root-like structure containing mycelium from the Lower Coal-Measures under the name of *Mycorrhizinium*. The fossil consisted of fragments of slender roots or possibly leafless rhizomes, the cortical tissues of which provided evidence of differentiation in respect to fungal infection, in itself an indication of mycorrhizal structure.

The distribution of mycelium was mainly intracellular with a local development of spherical and pear-shaped swellings or vesicles, and some evidence that intercellular growth of hyphae also occurred. Even more convincing evidence was provided by the structure of the middle cortical region, many of the cells in which contained dark-coloured structureless masses connected with the surrounding cell walls by radiating strands. The preservation of the tissues was fortunately sufficiently good to permit the identification of these strands as hyphae, and the general structure closely resembled that of corresponding cells containing mycelium in the "clumped" stage

of digestion in the endophytic mycorrhizas of *Psilotum* and many other plants (Pl. VII, figs. 36, 37).

Another fossil mycorrhiza was described five or six years later by Osborn (1909) in *Amyelon radicans*, the root of *Cordaites*, the well-known genus of Mesozoic Cycads. It had been observed previously that these roots often showed a development of clustered laterals of

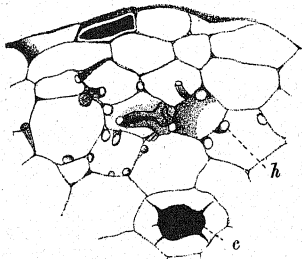


Fig. 48. *Mycorrhiza from the Coal-Measures*. Transverse section showing a portion of the external tissues of a fossil mycorrhiza. A cell of the exo-cortex contains fungal hyphae (*h*), and one of the cells of the medio-cortex a "clump" formation (*c*). (From Weiss, *Ann. of Bot.*, 1904.)

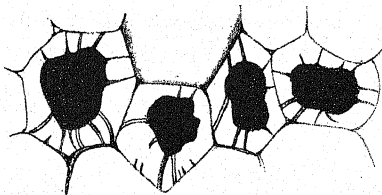


Fig. 49. *Mycorrhiza from the Coal-Measures*. Tangential longitudinal section through the medio-cortex of a fossil mycorrhiza. The cells contain mycelial "clumps" connected by hyphae with the cell-walls. (From Weiss, *Ann. of Bot.*, 1904.)

stunted growth, and Osborn's discovery that young rootlets from the clusters showed profuse infection of the cortical tissues by mycelium, led him to classify them with the "coralloid" roots so characteristic of living mycorrhiza plants. The localisation of infection to certain tissues and the absence of any apparent injury to the root cells also

pointed to their mycorrhizal character. Many cells of the inner cortex were filled with tangled skeins of mycelium, others provided clear evidence of intracellular digestion, while the formation of vesicles was a structural link with the fungi of recent mycorrhizas.

The general relation to the tissues of the hosts shown by the endotrophic mycelium in both these fossils, the evidence for simultaneous existence of intracellular hyphae in an active condition (Pilzwirthzellen) and similar hyphae in the course of digestion by root cells (Verdauungszellen), together with the morphological characters of the endophytes, constitute an irresistible case for regarding them both as endotrophic mycorrhizas of very similar structure to those formed by living plants. If this view is correct, the habit of forming root associations of a harmless or beneficial kind with soil fungi must have been well established in vascular plants as early as the Coal-Measure Period.

(To be continued)

PARASITISM IN THE GENUS *COMANDRA*

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(With 9 figures in the text)

THE genus *Comandra* is widely distributed in Canada, and is represented by four species, *C. umbellata* (L.) Nutt. and *C. richardsiana* Fernald in the eastern provinces¹, *C. pallida* DC. in the west, and *C. livida* Richards extending across the Dominion from Labrador and New Brunswick to British Columbia. *Comandra* is the "bastard toad-flax" of America, as is *Thesium* of Europe.

Like *Thesium*, *Santalum*, *Exocarpus*, and various other members of the Sandalwood Family, *Comandra* is a semi-parasite, being more or less parasitic on the underground parts of other plants, to which attachment is made by suckers or haustoria. Also, *Comandra* resembles other Santalaceous parasites in having a wide range of host plants. A few years ago Hedgecock (7) reported as hosts of *Comandra umbellata* some fifty species, representatives of thirty-one genera, including *Aster*, *Antennaria*, *Solidago*, *Rosa*, *Rubus*, *Fragaria*, *Vaccinium*, *Acer*, *Betula*, *Populus*, *Carex*, and various grasses. Quite recently, hosts of *Comandra pallida* have been reported (8) as follows: *Artemisia*, *Lupinus*, and *Achillea* (these in sage-brush lands), and the apple and peach trees. At Edmonton, Alberta, I have observed this species attached to roots of various grasses.

In regard to *Comandra richardsiana* and *C. livida* there seems to be in the literature no reference to the parasitic habit of these species. An examination of *C. richardsiana* in the vicinity of Toronto, Ontario, showed haustoria attached to a variety of host plants, including grasses, *Fragaria*, *Aster*, and *Rhus typhina* L. *Comandra livida* as observed by the writer at Banff, Alberta, was attached to roots of the lodge-pole pine (*Pinus murrayana* Balf.). Professor J. H. Faull and Mr C. G. Riley have examined this species of *Comandra* in northern Ontario and have found it parasitising the roots of the jack pine (*Pinus banksiana* Lamb.). Another host is *Ledum groenlandicum* Oeder. Slides showing the haustorium of *C. livida* attached to *Ledum* roots have been in the Department of Botany, University of Toronto, for several years. Through the

¹ The writer has recently discovered that *C. richardsiana* extends westward as a member of the northern coniferous forest.

courtesy of Professor R. B. Thomson I have had the privilege of examining these preparations, and have found that the haustoria resemble those on *Pinus* roots¹.

Hauستoria of several Santalaceous parasites have been described in greater or less detail by various writers. Among these descriptions, that of the *Santalum* haustorium by Barber (1, 2) is quite extensive and thorough. Recently, the mature haustorial attachment of *Comandra pallida* has been described briefly by Woodcock and de Zeeuw (10). My own studies, which have extended to *C. livida* and *C. richardsiana* and which include various stages in development, make possible a more complete account of the haustorium of this genus.

STRUCTURE OF THE MATURE HAUSTORIUM

The mature haustorium of *Comandra livida* is hemispherical to conical in shape, 2 to 5 mm. in diameter and 2 to 8 mm. in length. From the concave contact surface a sucker or process extends and penetrates the host tissues. A transverse section of a pine root, with an attached haustorium cut in the median vertical plane, shows a number of the salient features (Fig. 1). In the haustorium two regions may be noted, the *axial*, which at maturity consists largely of a core of tracheids, and the *cortical*, which is extended distally into two folds (clasping folds) that enclose the stem for a considerable portion of its circumference. On the inner side of the clasping folds, others (prying folds) have been thrust into the periderm of the host root. In various regions of the haustorium—in the middle of each prying fold, along the inner face of each clasping fold, and extending backwards towards the core—are the so-called "separation strips." These conspicuous layers consist of collapsed cells, and, as pointed out by Barber, are indicative of the series of changes in growth and pressure that have occurred in the haustorial tissues.

The origin of the "separation strips" has been considered rather obscure by certain writers. Two camera lucida drawings (Figs. 2 and 3) throw light on this point. Behind the growing tip of a young prying fold (Fig. 2) the median cells are compressed laterally and are finally completely flattened, thus giving rise to a layer of collapsed tissue. In an older and broader prying fold (Fig. 3) there is a record of a similar course of events. It is probable that the terminal and more active cells of the fold maintain a higher osmotic pressure than those which are left behind. Growth of the fold takes place by division

¹ Since the above was written, haustoria of *C. livida* have been found attached to roots of the following: *Picea*, *Ledum*, *Salix*, *Betula*, *Abnus*, *Ribes* and *Lonicera*.

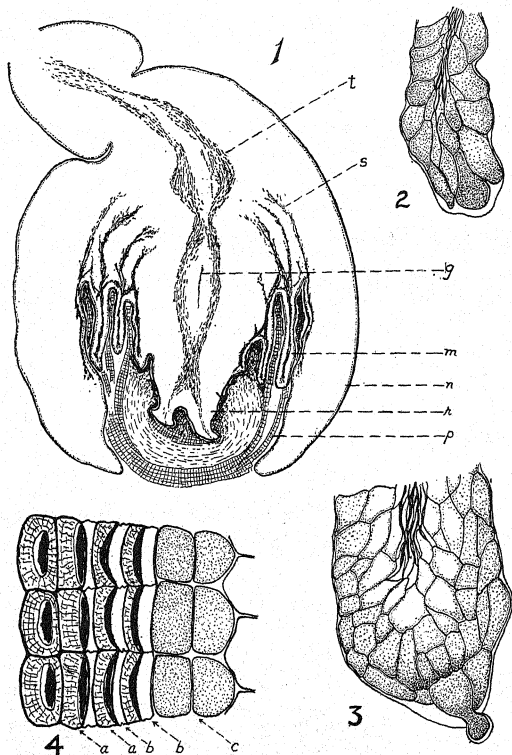


Fig. 1. Median vertical section of haustorium of *C. livida* attached to root of *Pinus*; *t*, haustorial tracheids; *s*, separation strip; *g*, slight break in tissue marking position of collapsed gland; *m*, prying fold; *n*, clasp; *r*, haustorial sucker; *p*, periderm of pine root.

Fig. 2. Tip of a young and narrow prying fold.

Fig. 3. Tip of a broader prying fold.

Fig. 4. Portion of periderm of *Pinus* root, showing layers of sclerotic (*a*) and cork (*b*) cells formed by the phellogen (*c*).

of elongated and rather bulbous terminal cells. The tip of the fold is bounded by a rather thick layer of a substance which is probably mucilaginous in nature. As is shown in Fig. 3, the expanding terminal cell may push through the bounding sheath, at the same time developing a similar sheath of its own. It is highly probable that the terminal cells of the prying fold function not merely in a mechanical way, but also effect chemical dissolution of the host tissues by the secretion of digesting substances.

It will be noted (Fig. 1) that the prying folds serve to push aside the periderm in thin layers, thereby facilitating the penetration of the haustorial sucker into the inner tissues of the host. As is shown in Fig. 4, the periderm of the pine root consists, in the main, of alternate layers of suberised and lignified cells, the latter being greatly thickened on the outer sides only. A layer of uniformly thickened sclerotic cells ordinarily occurs at the periphery of the periderm. Frequently, only one lamina of sclerotic cells is penetrated and turned back by a prying fold, for the latter follows the line of least resistance, namely, along a suberised layer. On parasitised pine roots several haustorial folds commonly occur, but on hosts whose bark is more readily penetrated fewer folds develop. For example, on roots of *Ledum* one pair of folds serves to penetrate and turn back the periderm, and, on the stem shown in Fig. 5 there is a very slight development of prying tissue.

The vascular system of the haustorium consists in the main of tracheids with reticulate thickenings. The tracheids extend from the xylem of the parent root, through the axial region of the haustorium, and to the tip of the sucker if the latter has reached the wood of the host. As has been described by Barber for the *Santalum* haustorium, the tracheids are frequently disposed in the shape of an hour-glass, or they may present a flask-shaped arrangement with the neck of the flask extending to the host tissues. Barber's detailed description of the vascular system is in general applicable to *Comandra*. However, certain differences seem to obtain, and these will be noted briefly here. Barber(2) gives a lengthy account of a peculiar "interrupted zone," occurring "slightly above the widest expansion of the hour-glass-shaped vascular loop," and in which "the vessels are all scattered, disintegrated and filled with gum." According to Barber this break in the vascular continuity is a regular phenomenon in *Santalum* and occurs also in the haustoria of some other plants of this type, but no explanation of its significance is offered. In my study of the *Comandra* haustorium I have not met

with anything suggestive of an "interrupted zone"; possibly a more careful examination of older haustoria would reveal it. Below the "interrupted zone" the xylem elements of *Santalum* are said to become organised into two broad bands. A similar grouping of tracheids in two strands characterises the haustoria of other Santalaceae hitherto described, with the exception of *Exocarpus*. In the latter genus, according to Benson(3), the tracheids are distributed over the surface of a central parenchymatous core. In *Comandra* also, a disperse arrangement of the haustorial tracheids occurs,

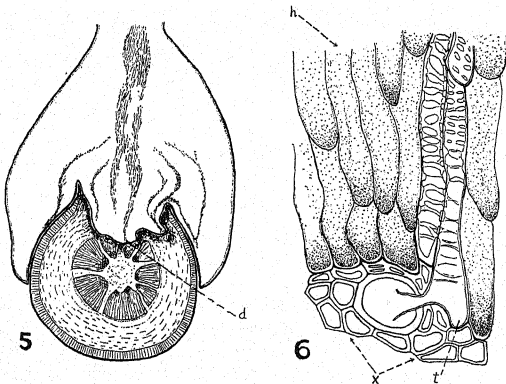


Fig. 5. Median vertical section of a haustorium of *C. livida*, attached to an underground stem of the same species—a case of self-parasitism.

Fig. 6. Detail of the region (*d*) in preceding figure, showing xylem elements (*x*), some of which are flattened by pressure of the haustorial cells (*h*); and a vessel which has been penetrated by the haustorial tracheid (*t*).

although in some parts of the axis there is no medulla but a single median plate of tracheids.

The foregoing description applies in particular to typical mature haustoria of *C. livida*. Many variations in form and habit occur in this species; indeed, almost all of the irregularities described by Barber have been observed. Among these may be mentioned cases of irregular penetration, distorted and asymmetrical forms, and the "compound" haustorium. The latter presents an appearance of two or more superimposed haustoria, and is produced in cases of repeated

attempts at penetration, by the development of new clasping folds from the axial region.

The mature haustorium of *C. richardsiana* was studied in considerable detail as it occurred on the roots of *Rhus typhina*. Numerous attachments were found on rather large roots, 5 to 12 mm. in diameter and 3 to 8 years or more in age. The haustoria measured 3 to 5 mm. in diameter and 2 to 3 mm. high, being rather flattened on top. One or two pairs of prying folds served to turn back the cork layer of the host root (Fig. 8), following which the haustorial sucker extended rapidly through the cortex and phloem to the xylem, the latter usually being penetrated to only a slight extent. Haustoria of this species, attached just below the surface of the ground to stems of grasses, developed several pairs of prying folds which served to penetrate and thrust aside the clasping leaf bases.

INTERRELATIONS OF HOST AND PARASITE

The interesting but very difficult subject of the mutual influence of host and parasite has been discussed at length by Barber and other writers on semi-parasites. For *Comandra* I have observed the usual indications of pressure (Fig. 6), as well as disorganisation and discoloration among the host cells, in the region where the parasite abuts on the host. Likewise, in this region, there is commonly a narrow, highly coloured zone which probably consists of enzymes, products of digestion, and protective gums. In cases where the woody cylinder is penetrated to some distance, fragments of partially digested xylem are sometimes surrounded by the soft tissues of the advancing sucker and appear as woody islands in the sea of haustorial parenchyma.

Roots of *Pinus* attacked by *C. livida* are frequently penetrated to such an extent that the vascular system is completely severed, and there seems to be no development of protective tissues by the host. Roots of *Rhus typhina* attacked by *C. richardsiana* are rarely penetrated beyond the last annual ring of the wood, and towards the close of the growing season a thick layer of cork is developed between the haustorium and the host tissues. A collection of this species made on August 1st showed only early stages in penetration and no sign of inward extension of cork around the haustorium, whereas a collection made early in October showed advanced stages in penetration and a peripheral development of cork, extending in some cases from the cortex into the xylem and not infrequently forming a complete barrier in front of the haustorial sucker. These

observations suggest that the reaction of the host tissues is merely a response to wounding and usually comes too late to retard the progress of the haustorium. In the following season, growth of the haustorium would be seriously impeded by the barrier of cork; indeed it appears that haustoria of *C. richardiana* on *Rhus* roots function for one season only.

The vascular connections of host and parasite take on an added interest in view of recent controversies concerning the functions of phloem and xylem as transporting channels. *Comandra* resembles other semi-parasites in that sieve tubes are absent in the haustorium, the only conducting elements being tracheids, which form a connection with the xylem of the host. These facts have led various writers to conclude that a haustorium of this type is concerned, primarily at least, with abstracting water and mineral substances. This conclusion is based on the belief, no longer tenable, that xylem transports water and inorganic substances only, whereas phloem transfers elaborated foods only. I have applied a number of micro-chemical tests with a view to throwing some light on the exchange of carbohydrates and other substances between haustorial and host tissues during the course of penetration, but have been unable to draw any general conclusions therefrom. It is probable that carbohydrates for the early growth of the haustorium (Fig. 7) are transported from the parent root, in part at least, through the primary haustorial tracheids. When vascular connection with the host is established, the haustorial tracheids effect transport, in the opposite direction, of water, mineral substances and probably organic materials as well. Of interest here is a fact emphasised by Benson (3), namely, that "the haustoria do more than just tap the water supply of foreign roots," for the attack is not limited to the conducting elements. Indeed, my preparations of *Comandra* suggest that organic substances are absorbed from various host tissues by the prying folds and particularly by the terminal thin-walled cells of the sucker.

Benson's account (3) of "phloeotracheides" in the haustoria of *Exocarpus* and *Thesium* is of interest. These conducting elements are described as lignified, lined with protoplasm but without nuclei, and containing numerous granules of a carbohydrate, probably amyloextrine. I have observed neither lining layers of protoplasm nor amyloextrine granules in the tracheids of the *Comandra* haustorium, and hence the term "phloeotracheide" cannot be applied here; however, this does not preclude the possibility of these elements functioning as transporting channels of carbohydrates.

The association of haustorial tracheids and host xylem ordinarily becomes very intimate. Many instances have been observed of a tracheid in close contact with a xylem element, and occasionally penetration of the latter seems to have been effected. One very clear case of this has been found (Fig. 6), in a stem of *C. livida* attacked by a haustorium of the same species. As shown in the drawing, the distal end of the haustorial tracheid is open and is located well within a host vessel. Lignification of the tracheid must have occurred subsequent to penetration. Since the relationship here is a special one, namely self-parasitism, one must not conclude that fusion of haustorial tracheid and host element is of general occurrence. For *Santalum*, Barber reports that in almost all cases there is the sharpest line of demarcation, generally accentuated by a layer of gummy substance, between haustorial sucker and host tissues; but, on the other hand, in certain cases of self-parasitism (haustoria of *Santalum* fixed to roots of the same species) actual fusion of tissues occurs. In passing it may be mentioned that self-parasitism is of widespread occurrence among Santalaceous and other semi-parasites.

DEVELOPMENT OF HAUSTORIUM AND PENETRATION OF HOST TISSUES

In Fig. 7 is represented a median vertical section of a young haustorium, at a stage before penetration of a foreign organ has commenced, in fact before clasping folds have been formed. At this time, the haustorium is typically somewhat pear-shaped and about 1 mm. in diameter. As shown in the drawing, the endodermis of the parent root opens out and extends into the neck of the haustorium, and tracheids become differentiated within the endodermis. Beyond the tracheids in the axial region, the cells are small, thin walled, filled with deeply-staining protoplasm and obviously meristematic, and constitute a tissue termed the "nucleus" by various writers on semi-parasites. Surrounding the axial region is the cortex, consisting of large, vacuolated, parenchymatous cells, many of which are commonly packed with starch grains.

The mode of origin and morphological nature of haustoria of this type have long been much debated subjects. Certain earlier writers regarded haustoria of root parasites as highly modified roots, while others contended that they are "emergences" or organs "sui generis." Critical investigations of more recent years seem to show that root parasites differ widely in this respect. For example, it is

said (9) that the haustorium of *Striga lutea*, a semi-parasite of the Scrophulariaceae, is markedly exogenous in origin, and shows no homologies with lateral roots. On the other hand, the haustorium of *Santalum* is not altogether the result of multiplication of superficial cells, for as Barber (1) writes: "the endodermis opens out where the mother root joins the haustorium, and it seems probable that the upper, proximal part of the haustorium is formed, as in *Thesium* described by Leclerc du Sablon, from tissues within the endodermal layer." *Comandra* appears to be very much like *Santalum* in this, as in so many other features. My observations indicate that the axial tissues, including tracheids and "nucleus," are descended from cells within the endodermis of the parent root, and that the cortex has developed from cortical cells of the parent root. If this interpretation be correct, the haustorium is a composite structure, the axial penetrating part being morphologically a root, and the surrounding cortical tissue a proliferation of the parent cortex.

In the young haustorium, two (or, in some cases, probably more) strands of tracheids come off from the xylem of the parent root. Farther down in the haustorium, these strands become divided into several, more or less distinct, small bundles, arranged around a central pith; and lower still, the tracheids become somewhat scattered in the axial region. At an early stage in penetration of the host tissues, a gland frequently appears in the lower part of the "nucleus" of the haustorium (Fig. 8). When penetration is well advanced, the gland collapses and the tracheids extend downwards, forming a loop around the glandular region (Fig. 1). Secondary thickening of the haustorial xylem commences at about this stage in development. Cambial cells appear outside of the primary tracheids, and, as a result of their activity, a cylinder or a core of secondary tracheids becomes organised in the upper part of the haustorium. Immediately above the gland, this core of xylem is much broadened in the direction of the longitudinal axis of the parasitised root. The general structure of the xylem in its mature condition has been described above.

The presence of a well-defined gland in the haustorium of *Comandra* is of considerable interest. Barber (1) reports glands in the haustoria of *Santalum* and other semi-parasites, and points out that Solms Laubach observed something of the kind in *Thesium*. In general, however, writers on Santalaceous and other semi-parasites have nothing to say about a gland in the haustorium. I have observed the gland in both *C. livida* and *C. richardsiana*. A collection

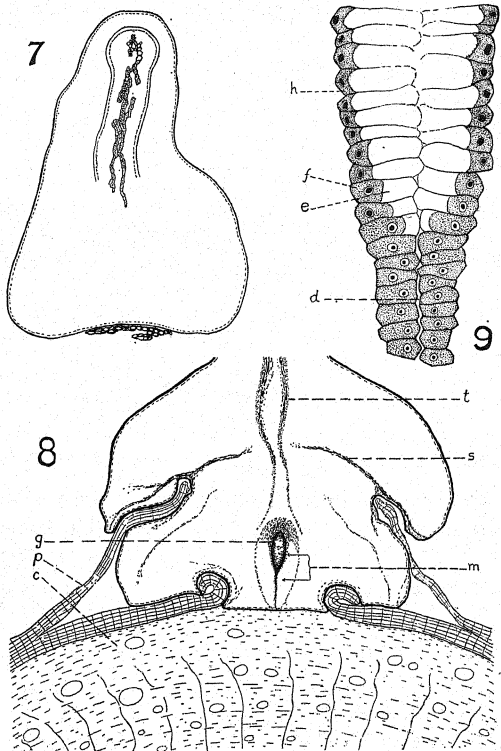


Fig. 7. Young haustorium of *C. livida*; haustorial tracheids connecting with the metaxylem of parent root; endodermis; fragment of host periderm clinging to the distal expanded end.

Fig. 8. Vertical section of haustorium of *C. richardsoniana* attached to root of *Rhus typhina*; *t*, haustorial tracheids; *s*, separation strip; *g*, gland; *p*, cork of host root; *c*, cortex.

Fig. 9. Portion (*m*) of gland in preceding figure; *d*, duct; *e*, secretion substance (unshaded parts of secreting cells); *f*, unaltered protoplasm at base of secreting cell; *h*, advanced stage in decomposition of protoplasm.

of the latter species made early in August showed glands in about 80 per cent. of the haustoria. The gland seemingly develops only when considerable resistance to penetration is experienced by the haustorial suckers, being absent where entry has been effected readily and quickly.

The gland arises endogenously in the "nucleus" of the haustorium subsequent to development of the claspings and first prying folds. At maturity it is somewhat rounded above, expanded in the central region and narrowed down distally to form a duct (Fig. 8). In the plane at right angles, the gland is considerably elongated. The secretory cells are arranged in two bands which meet in the median line, and at first are densely filled with deeply-staining, granular cytoplasm and have relatively large nuclei with conspicuous nucleoli. In the central part of the gland, these cells elongate and closely resemble in appearance the pyramidal secretory cells of various animal tissues. Large granules appear in the cells and the cytoplasm becomes gradually converted into a vitreous secretion substance. The latter does not stain with safranin and only faintly with Delafield's haematoxylin. The nucleus remains embedded in the unaltered granular cytoplasm at the base of the cell, and becomes gradually smaller, flattened and more deeply staining (Fig. 9). The distal end of the cell bulges somewhat, the wall is finally ruptured and the secretion substance escapes. In stained preparations, the secretion appears in the form of twisted filaments or curved rods, which are faintly coloured with haematoxylin. Before secretion commences, the cells draw apart more or less along the median line, forming an intercellular space. This glandular cavity, which thus arises schizogenously, is enlarged lysigenously by gradual disorganisation of the secreting cells. The shorter cells in the distal part also separate somewhat, forming a duct through which the secretion passes to the region of the host tissues.

DWARFED INDIVIDUALS OF *COMANDRA LIVIDA*

In the Banff district, Alberta, many dwarfed individuals of *C. livida* occurred wherever this species was encountered by the writer. These plants bore reduced leaves, more or less devoid of chlorophyll and varying from variegated to uniformly yellow in colour. Normal plants, vigorous in appearance and with larger green leaves, occurred in the same patches with the dwarfed individuals. An examination of a considerable number of plants indicated that a particular underground stem bore normal shoots

only or abnormal only. It is probable that this condition is general for *C. livida*. Brown and Schäffer(4), in their *Alpine Flora of the Canadian Rocky Mountains*, describe variegated plants of this species, and Dr J. H. Faull has observed them in northern Ontario. At Banff, I found these plants in three places, varying somewhat in altitude, nature of substratum, water supply and light intensity.

When these dwarfed plants were first met with by the writer, it was thought that they might possibly represent transitional stages in the development of a more completely parasitic habit. MacDougal(8) has concluded that "the evolutionary movement in the development of parasitism is generally toward increased dependence of the parasite accompanied by accentuated and more complete atrophy." If, perchance, dwarfing and loss of chlorophyll in *C. livida* are indicative of an increasing dependence of the parasite, we have here an extremely interesting case of evolutionary development. On the other hand, this condition may be due, as Brown and Schäffer suggest, to a fungoid or other disease of the plant.

Indeed, there is considerable evidence that these dwarfed individuals are affected by a disease of the mosaic type. Certain features exhibited by these plants—namely, marked reduction in leaf size, shortened internodes, mottling or complete yellowing of leaves, curling and ruffling of leaves and a curling downward of the leaf margin—are characteristic symptoms of mosaic diseases. Histologically too, the plants exhibit features typical of these diseases. In a recent paper, Dickson(5) concludes that certain microscopic modifications are common to mosaic diseases, at least to the sixteen forms which he examined. The more marked of these characteristics are as follows: (1) hypoplasia in lighter green areas; (2) reduction in the intercellular space volume of the areas and a tendency to muriform arrangement of the cells; (3) reduction in the chlorophyll content of these cells, due to fewer chloroplasts, less chlorophyll per plastid, or degeneration and general coalescence of plastids; (4) reduction of area covered by each epidermal cell, the latter being frequently deeper than normal; (5) in the deeper green areas of the leaves affected by the mosaic disease, a chlorophyll content greater than normal. A careful histological examination has revealed these features in the dwarfed individuals of *C. livida* and points to the conclusion that these plants are affected by a disease of the mosaic type; but final proof must await the results of experiments to determine the infectious nature of the juice of the abnormal plants.

SUMMARY

An account is given of the semi-parasitic habit and host plants of *Comandra*, with particular reference to *C. livida* Richards and *C. richardsoniana* Fernald. Parasitism of Coniferous (*Pinus*) roots by *C. livida* is of especial interest.

The haustorium, by which attachment is made to the underground parts of the host, is compared with haustoria of certain other semi-parasites. It bears a close resemblance to the *Santalum* haustorium described by Barber. The morphological nature of the haustorium is discussed.

Penetration of a foreign organ by the haustorium is effected by means of prying folds which thrust aside the periderm, and by a median sucker which pushes through the cortex and phloem to the xylem.

A well-defined gland frequently develops in the sucker of the haustorium, and probably functions in the production of digesting enzymes.

The haustorial tracheids become intimately associated with the host xylem. The question of the transfer of food materials is discussed.

Self-parasitism occurs in *Comandra* as in various other Santalaceous forms.

In various localities, *C. livida* is characterised by the occurrence in large numbers of variegated and more or less dwarfed individuals. Histologically, the leaves of these abnormal plants exhibit features which are typical of mosaic diseases.

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ON THE FORM OF THE PROTOPLAST IN CELLS OF THE GENUS *CERAMIMUM* AND THOSE OF *DASYA COCCINEA*

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(With 12 figures in the text)

INTRODUCTORY

WHEN I was engaged in examining the structure of the procarp in species of *Ceramium* and *Dasya* some years ago, I observed a curious condition of the protoplast in certain cells in these genera, and read a short paper thereon before the Botany section of the British Association at Bristol (1898). I supposed at the time that the condition I described had not been observed, or at any rate, figured, by any one else, but I subsequently found that Moore (1886) had already seen and figured it (as far as the genus *Ceramium* was concerned) in *C. rubrum* when he was investigating the continuity of protoplasm. Moore thought that Hick (1884) had also observed the same thing in *C. acanthonotum*, but a reference to Hick's paper does not appear to me to justify this view. However, Moore's observation seems to have been confined to a single species of *Ceramium*, and is disposed of in half a dozen lines in the text.

I was not able to follow up the study of this subject at the time, and it is only recently that I have been able to extend my observations among several species of *Ceramium*, and to make a more detailed study of the case of *D. coccinea*.

In the meantime another figure of the condition has appeared in a memoir by H. E. Petersen (1908) on Danish species of *Ceramium*, but I can find no reference to it in the French résumé, nor as far as I can make out, in the Danish text. An obscure indication of the condition occurs also in a figure in a recent paper (Grubb, 1925), but again without comment in the text. Beyond these, I have not found any reference to it in the literature, nor any figures of it, in the iconography of the genus.

With reference to *Ceramium*, I may state at once that the condition referred to is the existence of a strong trabecula of proto-

plasm, usually too coarse to be called a thread, running from pit to pit across the central vacuole in all the axial cells of the plant. It is present with some variations of structure in every species of *Ceramium* which I have yet examined. The condition in *Dasya coccinea* is of the same nature as that in *Ceramium*, but differs considerably in detail.

In the species of *Ceramium* in which an internodal gap is left in the cortication (what I may call the diaphanous series) it can be seen in all fresh and suitably fixed material without difficulty. Even in the completely corticated species (what I may call the *rubrum* series) it can still be seen when they are examined in bulk, but more faintly. When fixed material is treated with some protoplasmic stain, it becomes more apparent still, and can be traced through long series of cells without interruption, until it becomes lost in the less obviously vacuolated cells in the apical region. The trabeculae are seen best of all, in material which has been cut longitudinally by means of the microtome.

Whether these trabeculae can be found in herbarium material seems to me to depend largely upon the conditions under which it has been dried. In the more delicate diaphanous forms, the trabeculae disappear more quickly by disorganisation than they do in the coarser corticated forms. I should think therefore that the former would have to be dried promptly from the fresh condition to preserve the trabeculae intact and then mounted without crushing. But in the coarser forms they are less easily damaged, and I have been able to find evidence of their occurrence in very old herbarium material.

It seems to me impossible that this condition has not often been noticed by other observers. I can only conclude that, being mostly intent on other matters, they have passed it by as a curiosity of protoplasmic structure of no particular significance. I think however the existence of these trabeculae will be found to be inseparably associated with the structure and function of the characteristic floridean pit, and will therefore prove of interest both to the histologist and physiologist. I might also cite a case in which the trabecula may even become a matter of interest to the morphologist and systematist. In Harvey's *Phycologia Britannica* there stand on either side the genus *Ceramium*, as its nearest allied genera presumably, *Microcladia* and *Spyridia*. From a previous study of *Spyridia*, I knew that no trabeculae existed in *Sp. filamentosa*, and on other grounds I was inclined to regard that genus as remote from

Ceramium. But what about *Microcladia*? From all the descriptions of *M. glandulosa* (which I have never seen in the living state) one finds it difficult to see why it should ever have been given separate generic rank from *Ceramium*. This doubt is surely confirmed when by the examination of a scrap of *M. glandulosa* from the *Algae Damnonienses* I was able to show that the trabecula is as marked a feature of that plant as it is of *C. rubrum*.

I propose therefore to describe and figure the coarse features of these trabeculae in a number of species of *Ceramium* which I have been able to collect on the shores of Anglesey, and afterwards deal with the case of *Dasya coccinea*. I do not propose to enter upon a discussion of the minute structure and function of the floridean pit, though as I have said, I believe the question is ultimately bound up with that of these trabeculae. Nor on the other hand do I intend to attempt to unravel the intricacies of the forms in the genus *Ceramium*, by which the student of the genus is met on the very threshold of the subject. I will venture however as occasion arises to make some passing comments on both these matters. And later I hope to make some contribution to the study of the various forms of *Ceramium* by comparing the plants growing in our local waters with those familiar to collectors elsewhere. My material has been mostly examined fresh and in bulk, and when it has been necessary to do more, I have been content to examine material fixed in Flemming's mixture, and sectioned by means of the freezing microtome.

I. THE DIAPHANOUS OR INCOMPLETELY CORTICATED SERIES OF *CERAMIMUM*

1. *Ceramium ciliatum* Ducluz.

Although this species is not reported by Holmes and Batters from this area, a plant collected in November at Rhoscolyn, Anglesey, comes nearest to it of all the four spiny species figured in the *Phycologia Britannica* (1846-1851). As these four species remain in Holmes and Batters' List drawn up much later (1892) with the addition only of a form *decurrens* of *C. acanthonotum*, one would have supposed that in the group *armatae* therefore a fair degree of stability had been reached. I have not however found it so, for in this first form which I selected as promising to illustrate well the trabecula which I am about to describe, I found a plant which it seemed difficult to place, with Harvey's description before one.

In the first place Harvey depicts internodal clear gaps as existing close behind the apex (Fig. 1), while in this plant they do not begin to be apparent until the 3rd or 4th forking (about 50 articles) behind the apex (Fig. 3). Further Harvey shows at least a dozen spines in one circlet at each belt, in this plant there are steadily 7 throughout the plant. Johnstone and Croall (1860) certainly speak of *C. ciliatum* as having 6 to 8 spines, but like Harvey show diaphanous internodal gaps close up to the apex.

I may state here that I regard the number of primary spines as a sound criterion for diagnosis, as it corresponds to the number of pericentral cells cut off from the axial cell in the apical region (Fig. 2). We know how useful this latter character has proved for specific diagnosis in the genus *Polysiphonia*, and because it is not readily ascertained in the genus *Ceramium* as in *Polysiphonia*, it does not follow that it is not an equally trustworthy criterion in both genera. However, in the plant now under consideration it is readily ascertained, as each pericentral gives rise to one spine and one only. Where secondary spines arise as in *C. echionotum*, the primary spines which are first produced correspond strictly to pericentrals, though in that species all the pericentrals do not necessarily generate primary spines. The secondary spines seem to arise on any cortical cells, and they are usually much smaller than the primary spines. In *C. echionotum* the number of pericentrals is steadily 7, as far as I have been able to ascertain.

Now in Harvey's figures of *C. ciliatum*, in one figure the spines are as many as a dozen, in another bearing tetraspores there seems to be exactly 7. But the production of tetraspores cannot affect the number of spines, as the spines are borne close up to the apical cell, and the tetraspores are differentiated considerably later. I am inclined therefore to believe that two forms are depicted in Harvey's plate, of which one only is Johnstone and Croall's plant, and identical with the one here considered. It might be called the 7-spined form (Fig. 2).

The trabeculae may be seen in fresh material even in the younger parts where the internodal gaps have not yet appeared running without interruption for great distances. At each forking a trabecula takes the form of the letter Y, each of the three arms being of about equal length. Where the internodal gaps widen they may be seen in bulk much more clearly, as there is then no belt of cortical cells to obscure the view, excepting at the ends of the axial cells. Trabeculae may also be detected in the primary pericentral cells, but I have not been able to trace them in the other cortical cells.

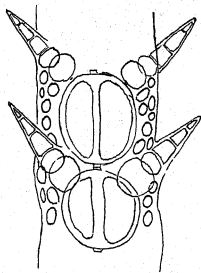


Fig. 1

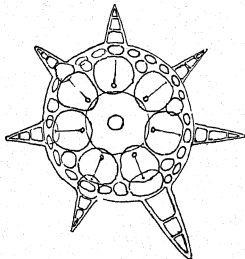


Fig. 2

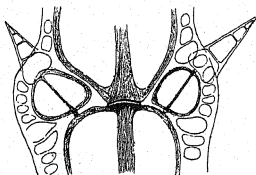


Fig. 4

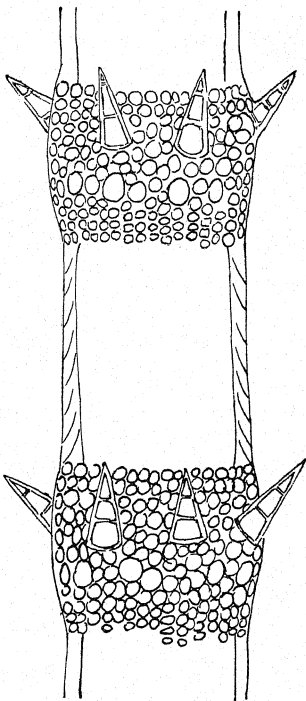


Fig. 3

Ceramium ciliatum, 7-spined form

Fig. 1. Longitudinal section of 2 axial cells from the apical region before the diaphanous gap in the cortex has arisen.

Fig. 2. Transverse section through a node showing the 7 pericentral cells, and the 3-celled spines arising from each.

Fig. 3. Appearance of the axis after the appearance of the diaphanous gap, showing by the striation of the wall the insertion of new layers in basipetal succession, and the sharp upper edge and irregular lower edge of the cortical belts.

Fig. 4. Appearance presented at the pit in well-preserved Flemming-alcohol material, on longitudinal section after freezing. A granulation of stained matter may be detected between the discs, but not connecting threads.

They run in all cases from pit to pit and hold an almost straight course, through the middle of the large vacuole of the axial or pericentral cell.

They are not mere threads of protoplasm such as are often found in vacuolated cells, but even in young cells seem solid plugs or pillars of protoplasm laden with granular matter like the endoplasm of the utricular portion of the protoplast, and in this plant are never less than 10μ thick. The granular matter seems to consist of three kinds of granules, (a) the finer granules which abound in the utricle, (b) starch granules which are much larger, and (c) still larger polygonal bodies which I take to be crystalloids. I do not doubt that streaming of the protoplasm occurs in these trabeculae, but I have not been able to demonstrate its occurrence in any species even when recently gathered. The movement is probably slow at best, but it ought to be demonstrable under favourable conditions.

Nuclei are not easy to discover in these axial cells of *Ceramium*, but nucleolated bodies may be found in pairs in the utricular protoplasm near the base of the trabeculae, and these I take to be the divided nucleus. But I have not found that the nuclei enter the trabecula. The chromatophores are of course confined entirely to the parietal ectoplasm of the utricle.

A remarkable feature of these trabeculae is that as they become older, there arise within their substance smaller vacuoles which in time melt together until the solid plug becomes a cylinder, and we have, as it were, a vacuole within a vacuole (Fig. 5). In an early stage of this vacuolisation, the elliptical space suggests an imbedded nucleus and such at first I took it to be, but as more than one such space appears and the whole trabecula gradually becomes a tube, the structure explains itself. The granular bodies that roughen the outside of the trabeculae project equally into the cavity of the trabecular vacuole (Fig. 6). In properly fixed material longitudinal sections of the cylindrical trabeculae may be obtained.

Before I proceed to describe how these trabeculae are related to the pit, it is necessary to state briefly how the appearances in the pit itself have been explained, say by such an observer as Schmitz. First there is the exceedingly thin closing membrane (*Schliesshaut*), the real boundary between cell and cell, corresponding in situation to the middle lamella elsewhere. Next closely applied to this are two circular discs (*Platten*), each disc with marginal slightly thickened rim, imbedded at their periphery in the cell wall. On account of the high refrangibility and sensitiveness to haematoxylin staining the

discs are readily made out, but the closing membrane for most observers has only a hypothetical existence as a discontinuity between the discs. With the surface of the discs the protoplasm of the pit is closely incorporated, so much so that the discs seem only an altered portion of the protoplasm, and are only with difficulty separable from it. On the other hand the two discs are readily widely separable from each other by various reagents as the result of what appears to be the dissolution of the closing membrane (Fig. 4).

Whether there is continuity of protoplasm at the pit between cell and cell depends first upon what happens at the discs, whether there are perforations into which the pit protoplasm passes, and then upon what happens at the closing membrane, whether the threads of the perforations of the discs pass also through this. Schmitz believed this continuity existed beyond doubt. This is indirect continuity, not direct continuity by means of one solid strand such as Hick believed in.

I am only concerned here with the relation of the protoplasm of the trabeculae to that engaged to the surface of the discs. The protoplasm of the disc surface of any ordinary cell where no trabecula occurs, is obviously different in character from the rest of the protoplasm. It is laden with matter which stains more deeply with reagents, and it seems somewhat fibrous with the fibres standing out from the disc surface like a fur. When a trabecula occurs, as in the axial cells of *Ceramium*, this fibrous or striated condition continues into the trabecula for a considerable distance, while it does not spread out similarly into the parietal protoplasm of the end of the cell. Indeed the whole appearance of the trabecula is that of a column with each end firmly fixed on to the surface of the disc. When plasmolysis occurs in these axial cells, the protoplasm comes away from the outer and end walls freely, but it always remains attached to the surface of the discs, which themselves retain their position at the bottom of the pits. The utricle thus assumes a characteristic appearance as of a collapsed puckered bag narrowing to both ends, in which however the trabecula may still be distinguished. On treatment with chlorzinciodide the discs move apart, and are carried with the protoplasm of the pit into positions considerably distant from their point of origin.

In fixed material sectioned after freezing, I have frequently found that the trabeculae seem to break with a fracture unlike ordinary protoplasm, though how far this was due to a difference in the texture of the protoplasm, and not to the freezing process, I could not decide.

In this species I have made out that the axial cells as they elongate grow at the lower end and not at the upper end. I give a figure (3) showing the layering of the wall and the insertion of new layers at the base. It is in keeping with this structure that the wall when it is gradually tinged with Delafield's haematoxylin the staining begins in the older region near the top and gradually extends downwards towards the newer parts. I have no doubt that it is in consequence of this structure that the lower margin of the nodal cortical belt grows more freely downwards than the upper margin grows upwards.

2. *Ceramium echionotum* J. Ag.

This was a plant collected at Rhos Neigr, Anglesey, in October. Again it does not seem to have been reported from this section of the coastline, and again I have not found its identification to be easy. *C. echionotum* is the only species with a 1-celled spine, and were that all, ought to be easy of determination, but this plant differs so much from that figured by Harvey, that one is disposed to agree with him, that more than one species may still be confounded under the name. Whereas the internodal gaps in Harvey's plant begin to appear close behind the apex, in this they only begin to appear after the 4th forking, and it is only after the 6th forking that they become about three times the width of the nodal belt, at which they afterwards remain. Then again, the spines in the young parts only appear on the outside of the arches, and in three rows giving the plant the appearance figured by Harvey for *C. flabelligerum* which however has a 3-celled spine. Harvey's typical *C. echionotum* seems equally spined on all sides. However, smaller secondary spines (not correlatable with pericentral cells) do occur but not to the extent figured for the typical *C. echionotum*.

The trabeculae are large, coarse, charged with granules, and easily seen through the cortical layer even before the internodal gaps appear (Figs. 5 and 6).

3. *Ceramium strictum* Kutz, collected at Rhoscolyn and Rhos Neigr, Anglesey, in October and November

I found this beautiful plant growing in abundance at Rhoscolyn in October on leaves of *Zostera*, fringing them with a border of bright red colour three or four times the width of the leaf. It has the diaphanous internodal gaps close to the apex, but it has not the adventitious branches (lateral ramuli) of *C. diaphanum* and *C. gra-*

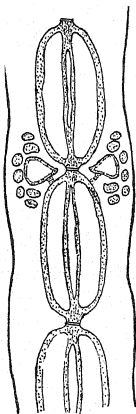


Fig. 5

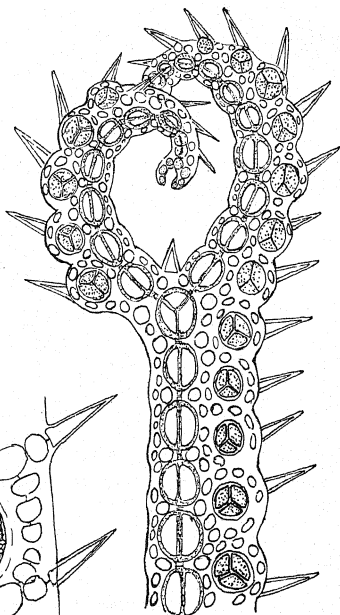


Fig. 6

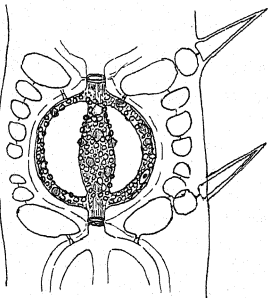


Fig. 7

Ceramium strictum

Fig. 5. Three axial cells showing vacuolisation of trabeculae.

Ceramium echionotum

Fig. 6. Longitudinal section of a tetrasporiferous plant in the apical region before the origin of the diaphanous gaps.

Fig. 7. A single cell in longitudinal section showing nucleus, starch granules, and crystalloids, and the distension by vacuolisation of the trabecula.

cillimum. In these respects it is more like *C. strictum*, with which it also agrees in being beset with hairs on the younger parts. It is smaller in size than *C. diaphanum* as figured by Harvey, being about 2-3 inches long as against the 6-8 inches of the latter plant.

It is an excellent plant in which to observe the trabeculae of the axial cells. The axial cells grow at the base as I have described under *C. ciliatum*. The upper margin of the cortical belt in this species is a sharp transverse line where the lower edge is somewhat irregular. As the axial cells elongate, so does the trabecula, until it becomes much attenuated in older cells, and I believe that the attenuation is the greater at the basal end of the trabecula.

When this species deteriorates by being kept in the laboratory, as it is liable to do on account of its delicacy, it is possible to observe the gradual disintegration of the trabecula leaving a trail of granular matter within the vacuole. Sometimes it sags over to one side of the cell until it ultimately falls against the wall. In this disintegration the crystalloids swell up until they take on the appearance of small vesicles attached to the trabecula.

This species has only five pericentral cells. Both tetraspores (about three at each belt) and antheridia are produced close up to the apex, and it is a noticeable feature that the antheridia are not produced simultaneously all round the belt. Often it would appear as if only a portion of the circumference (the outer) becomes antheridiferous at all. The downward growth of the chromatophores in the elongating is an interesting feature to observe. They assume a moniliiform appearance, and each bead seems to contain a well-defined granular body in the ordinary stroma. One hesitates to call it a pyrenoid in the absence of any definite relation to the granules of starch.

The nuclei in this plant, when it is examined in bulk, do not seem so susceptible to haematoxylin stains as they are elsewhere. However, I have repeatedly found nucleolated paired bodies at the base of the large axial cells which I take to be nuclei after division.

II. THE RUBRUM OR COMPLETELY CORTICATED SERIES OF *CERAMIUM*

The three completely corticated British species of *Ceramium* enumerated by Harvey are *C. rubrum*, *C. botryocarpum* and *C. flabelligerum*. To these, as I have already said, it seems to me that *Microcladia glandulosa* might well be joined. Of these *C. botryocarpum* disappears in Holmes and Batters' List as a species, and reappears

as a form *prolifera* of *C. rubrum*, the clumped fructifications which occur on tetrasporiferous plants being regarded as paraspores, and comparable with similar structures occurring on other species of *Ceramium*, e.g. *C. Deslongchampsii*, and there also on the tetrasporiferous plant. I have not yet found the completely corticated and spiny species *C. flabelligerum* in this area.

I have examined a great number of plants of *C. rubrum* differing greatly in size, habit, and degree of cortication in the apical region, but have not attempted a systematic study of the various forms. Petersen (1908) believes that many of the forms occurring on the shores of Denmark owe their existence to the varying degrees of salinity of the Danish waters. As I have only collected on shores facing the open sea, that factor cannot account here for the great multiplicity of forms.

To what extent the number of the pericentral cells occurring in a form can be made use of as an additional criterion in diagnosis, I am as yet unable to say. I feel sure that the number is invariable for the same plant but whether it varies sufficiently from form to form to be of much value I have not yet made out. The usual number is 7, but I found plants with only 6. In counting the pericentrals care has to be taken, as I have found that in older plants a derivative of a pericentral may sometimes grow inwards and so wedge itself into the ring as to give the appearance of an extra cell, and it is only when the pit connections are traced that the number can be correctly counted. It is noteworthy that, notwithstanding the facility with which secondary pits are formed in many Florideae, no secondary pits are ordinarily formed in the axial cell of a *Ceramium*. It has usually its own two genetic pits, plus those with its pericentrals and no more.

With regard to the trabeculae (Fig. 8), they never fail in any of the forms, and it is in this aggregate that the other observers, Moore, Petersen, and Grubb, have given indications of them in their figures. In all of them the solid plug of protoplasm seems sooner or later to become a cylinder as indicated by Moore and Petersen (Fig. 6). The single lenticular nucleus of the axial cell is parietal in position, and never occurs, as far as I have seen, in the trabecula. I have not seen the evidences of division of the nucleus, which seemed to me to occur in some of the diaphanous species.

As the axial cells in some forms of the *C. rubrum* series grow in the older parts to be several times as long as broad, I was curious to ascertain what changes took place in the trabecula in these

long cells. I cannot find that it ever breaks down completely, as it seems to do in some of the diaphanous species, but becomes attenuated into a long thin cylinder which can only be followed even in stained material by careful focussing. Whether in this group it ever

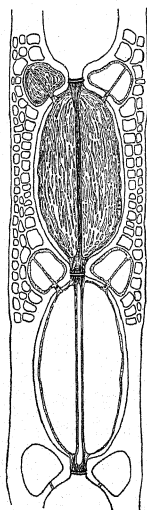


Fig. 8

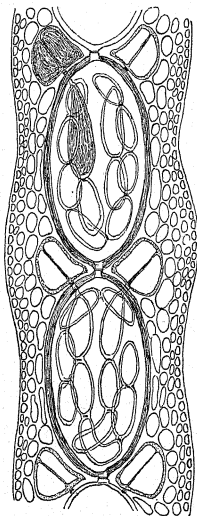


Fig. 9

Ceramium rubrum, a form of

Fig. 8. Two axial cells in longitudinal section from the basal region of a robust form. The upper joint shows the apparently solid trabecula. The pericentral cells also possess trabeculae. The chromatophores are imbedded in the utricle (slightly withdrawn from the wall) and are elongated sinuous structures running longitudinally.

Fig. 9. Showing the irruption into the cell cavity of old cells of filamentous growths entering by the pits connecting axial and pericentral cells.

becomes inflated into a delicate sac, as appeared to me to be the case in some diaphanous species, I have not been able to ascertain. I have found material cut in paraffin very difficult to interpret in these investigations

It was while I was examining these older axial cells that I happened on the condition represented in Fig. 9, to which although it is beside the topic under consideration, I must on account of its exceptional character, devote a paragraph. The material was well preserved, having been originally fixed in Flemming's mixture. I thought at first that the appearance was due to the invasion of the cavity of the axial cell by some parasite, but I soon discovered that the chromatophores of the supposed parasite were exactly like those of the host itself, and that the mode of entry was usually by means of the pits connecting the axial cell with its pericentrals. It is in fact a phenomenon like that described as tylosis in vascular plants, where a living cell bordering a pitted vessel sets up such a hydrostatic pressure on the pit membrane as to cause it to bulge into the cavity carrying with it a portion of the utricular protoplasm, and ultimately forming therein a thin-walled parenchymatous tissue, not infrequently filling up the whole cavity. The difference here is, that ingrowths are purely filamentous. There is no doubt about the entry through the pits, for some of them may be detected in the act of entry. But I have also found that these irruptions into the axial cells may also arise from other adjacent cells, and particularly the pericentral cells of the node next below, with which secondary pit connections must therefore be formed. This phenomenon seems to suggest that these older axial cells have lost vitality, and have no longer a hydrostatic pressure to balance that of the pericentral cells, in which case their function is chiefly mechanical. It is difficult to say whether or not these incursive filaments are of value as assimilating tissue, for while they contain plenty of chromatophores, they must be somewhat removed from the influence of the light (Fig. 9).

III. *DASYA COCCINEA* C. AG.

I will now describe the analogous condition in *D. coccinea*. As is known from Kny's researches, the axis of this plant is a sympodium. While the sympodial axis becomes corticated by longitudinal division of an axial cell much in the same manner as a species of *Polysiphonia*, the terminal portions which are thrust aside by the development of the sympodial axis remain, except for the basal joint, in an uncorticated state, like branches of a *Callithamnion*, and ultimately after branching once or twice exhaust their capacity for apical growth and terminate in fine pointed cells. The cells of these filaments are rather large and it is in these that the protoplasmic threads occur.

The cells of these filaments vary considerably in the quantity of starch granules and crystalloids which they contain. In order to see the threads, it is desirable to search for filaments with the minimum of the products of assimilation (Fig. 10).

The cell includes a large central vacuole. Forming a tessellation near the wall imbedded in the utricular protoplasm are the lenticular chromatophores. In the endoplasm will be found even in the poorly stored cells clumps of starch grains and crystalloids projecting somewhat into the cell vacuole.

Running straight from pit to pit across the middle of the vacuole will be found a fine thread of protoplasm, and as the thread reappears in every cell from base to apex of the 14- to 18-celled filaments, it is in stained material a striking feature in their histology.

Careful focussing will however discover that these threads are not always unbranched. The main thread runs fairly straight, but subsidiary threads may be seen to arise from it and to run sometimes at an angle, mostly obliquely, to the utricular protoplasm and usually to the nearest point of a clump of starch granules. Occasionally the main thread may be slightly bent at the point where a lateral thread is given off.

By a process of clearing away as far as possible the starch granules, and subsequent haematoxylin staining, it can be shown that each clump of granules represents the site of a nucleus imbedded in the ectoplasm (Fig. 11). It is difficult to remove the large crystalloids belonging to the clumps, which also take up the stain freely. But the sharp nucleolus, and the clear nuclear membrane mark the nucleus clearly.

When the sympodial axis of *D. coccinea* gives rise to a cortex, it does so after the manner of a *Polysiphonia*, that is to say, pericentral cells are cut off in succession (in this case 4 at the peripheral end, and 6-8 farther back) each of which is from the first equally long with the axial cell. These pericentrals in their turn give rise to another ring of secondary pericentral cells, and the axis has by this time reached a considerable degree of thickness.

Where the monosiphonous condition of the filaments passes into the polysiphonous condition of the axis, it is easy to see that the protoplasmic thread of the lowest cell of the filament passes into the pit of the adjacent axial cell. As I was curious to know whether the thread continued into the axial cells, I cut longitudinal sections of the axis a little way back along the sympodium, and was interested to find that the central cells each possessed a solid trabecula

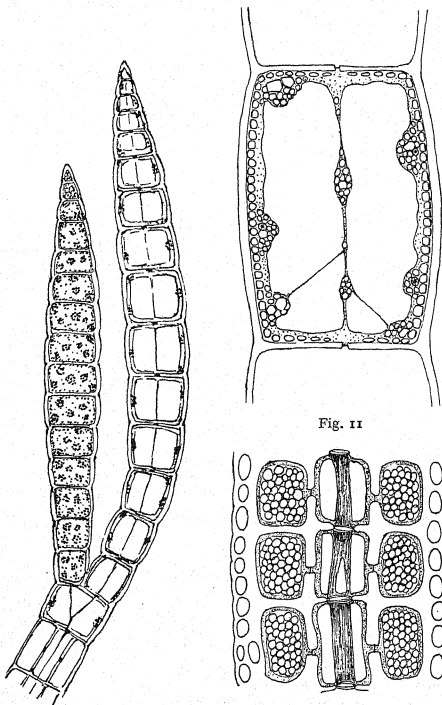


Fig. 10

Fig. 11

Fig. 12

Dasya coccinea

Fig. 10. Uncorticated terminal portions of sympodial axis, showing on the left the appearance at the surface, and on the right the axial threads running from pit to pit.

Fig. 11. A single cell showing axial thread and lateral connections. The chromatophores form a tessellation at the surface. Each clump of granules contains a nucleus, starch granules, and crystalloids.

Fig. 12. The trabeculae in older corticated portion of the sympodial axis.

passing from pit to pit of much the same appearance as that of *Ceramium*. The central cell is here a cylinder and not a barrel-shaped body as in *Ceramium*, and the trabecula thus occupies a larger share of the diameter. The trabecula is laden with fine granules, but the axial cell is clearly not a place for storage. That function belongs to the pericentral cells which are chockfull of starch and other granules. The secondary pericentrals are again assimilatory, with conspicuous chromatophores. In fact, the axis of *Dasya* illustrates well at this point three of the physiological tissue systems which Wille found in Florideae. Axial cell, pericentral cells and secondary pericentral (surface) cells stand respectively for transport, storage and assimilation. The fourth system of Wille is provided for farther back on the sympodium by the strengthening hyphae which grow downwards among the surface cells (Fig. 12).

THEORETICAL CONCLUSIONS

Transport of elaborated substance from part to part seems to be the special function of these protoplasmic strands whether they be threads or, as I have called the coarser threads, trabeculae. The direction of transport is doubtless from the place of elaboration of organic substance or its storage to the region where it is being required for growth and reproduction, and thus it may vary in the same transporting tissue from time to time.

There is also another possibility, the transference of stimulus from part to part. It is suggestive that the main threads which run from pit to pit in the filaments of *Dasya* send off side threads to the spots where the nuclei lie in the parietal protoplasm, and where the products of assimilation are being piled up. The stimulus which the zygote seems to communicate to neighbouring cells, whether for the formation of a fruit wall or for its own development of gonimoblast filaments and carpospores, must pass along the protoplasm, and these strands, which form short cuts from pit to pit, seem a suitable means by which the stimulus may pass.

The protoplasmic continuity through the walls and pits of contiguous cells in the higher plants, has been generally accepted as serving the double purpose of transference of organic substance, and convection of stimuli. The Florideae are plants where the connection between cell and cell is often confined to a single pit, which therefore is larger than ordinary, and of more complex structure. Its functions however remain the same.

The intimate connection of the trabecula with the discs of the pit in *Ceramium* and *Dasya* seems to suggest that the trabecula serves also to promote the functions of the pit, in fact that it is part of one organisation for rapid transport (Fig. 12).

It will be remembered that Nemec (1900) found, in cells of the root apex of the higher plants, fine fibrillae imbedded in the cytoplasm, which he considered were the special organs of transmission of stimuli between the perceptive root tip and the curving region behind the tip. The trabeculae of *Ceramium* and *Dasya* would seem to be fascicles of such fibrillae, and suggest rapid convection of stimuli.

The vacuolisation of the trabecula in *Ceramium* is an interesting phenomenon, as it really involves a vacuole within a vacuole. There is probably some difference of density between the sap of the inner and outer vacuoles, and osmotic interchange may so exist that the vacuole of the trabecula may distend or collapse again. It naturally suggests itself to anyone looking at such a figure as Fig. 4, that as the internal vacuoles form a chain separated by the pits, the concentration of their sap may be higher than that of the external vacuoles, and the dissolved substances may more rapidly pass along the filament, than when dissolved in the larger volume of liquid in the outer vacuole. But these are matters demanding closer inquiry than I have been able to give to them.

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A REPLY TO COMMENTS ON THE THEORY OF THE SOLID CARPEL AND CARPEL POLYMORPHISM

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(With 6 figures in the text)

IN the article entitled "Comments on the Theory of the Solid Carpel and Carpel Polymorphism," by John Parkin, which appeared in the last issue of this journal¹ the writer sets out, as he tells us (p. 191), to attempt a critical review of the above theory which, in his opinion, "imposes on the student a greater difficulty than there is at present in grasping the structure of such [= syncarpous] gynoeceia" (p. 192). After reading these lines I was prepared to find that the writer had a new and convincing interpretation to offer of the numerous morphological facts which are generally acknowledged to be difficult to harmonise, and in some cases even to be definitely at variance with the currently accepted view of one uniform type of carpel fulfilling all the carpellary functions, but which I have elsewhere² endeavoured to show fall into line and present no difficulty once the polymorphic character of the carpel is accepted with its consequent implications.

But in what follows these opening remarks one looks in vain for any real contribution in the way of fresh observation or any convincing interpretation of these (from the writer's standpoint) decidedly awkward facts of which, from the above citation, it is nevertheless clear that he is not unaware. As he indeed again shows by his acknowledgment that had I restricted the theory of carpel polymorphism to the Cruciferae he would have been disposed to accept it. But since I have attempted to show that it must be accepted as applicable generally throughout the Angiosperms he decides that it is more prudent to stop while he yet may, and without any serious attempt to tackle the evidence on which the theory rests he turns back from the conclusions which he sees taking shape ahead, and

¹ *New Phyt.* 25, p. 191.

² *Ann. Bot.* 37, p. 451, 1923; *ibid.* 39, p. 123, 1925; *J. Roy. Hort. Soc.* 50, p. 241, 1925; and *New Phyt.* 24, p. 206, 1925.

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pronounces for the old traditional explanations. Yet had the conception of polymorphic carpels been put forward as a solution applying only to the single family of the Cruciferae it could have had no value—save apparently in the eyes of my critic. The problems presented by the Cruciferae cannot be treated wholly apart and without reference to those of like nature in other families. Any solution holding good for the typical Crucifer must obviously be capable of application to Papaveraceae of the type of *Chelidonium*, to the Fumariaceae and to the section Cleomoideae of the Capparidaceae. But the same kind of polymorphism as is met with in these cases occurs throughout the families to which they belong, and what is found to be general throughout the Rhoeadales can be paralleled in other Orders. The same carpellary relations demand the same explanations, and the writer gives his case away hopelessly once and for all when he admits that the theory appears to meet the case of the Cruciferae. That granted, the rest follows. We have indeed in its very wide application not, as the writer would imply, grounds for looking askance at the conception of carpel polymorphism but, on the contrary, the strongest possible argument in its support. At a later point (p. 201) the writer makes another frank admission. He tells us "that he has not to any extent examined for himself" the material which I have employed. Surely an amazing confession! The more so that much of the material in question lies to hand everywhere, and that no special technique is required. A rough slice cut with a penknife through the base of the ovary of Tulip or Lily should suffice to arouse doubt as to the correctness of the current interpretation in an open mind, once the question has been raised. But apparently sooner than risk the upset of a cherished view by observing the facts at first-hand the writer is content to pen a plaintive protest from his study. His first objection (p. 192) is to the use of evidence derived from abnormal structures. I have met this curious attitude before and this circumstance led me in an earlier account¹ to draw attention to de Candolle's pronouncement as to the immense value of such evidence in connection with this kind of problem, and I might emphasise it again. If de Candolle's conclusion, reached after much study of this type of structure makes no appeal I can scarcely hope that the evidence of this nature which I have adduced will weigh with those who take my critic's standpoint. Yet the facts are sufficiently striking.

Let us take the case of a 4-valved stock fruit. This abnormal

¹ See "Carpel Polymorphism I," *Ann. Bot.* 39, p. 167.

type of siliqua has all the characters that we associate with normality except for the increase in the carpel number. Furthermore these exceptional fruits do not occur entirely at haphazard. They are found in a definite region of the inflorescence; in that region, to wit, in which, if the phenomenon occurs at all, we should expect ontogeny to repeat phylogeny, i.e. at the base of the flowering axis¹. The distribution is even more strictly localised in other instances which I have cited, as e.g. the exceptional many-styled pods of *Eschscholzia* and the peculiar urn-shaped fruits of *Ceratocarpus* (*Corydalis*) *heterocarpa*. Is there any reasonable ground for ignoring the significance of these facts? Can we attribute any other significance to them than that of reversion to an ancestral form? Again, except on the supposition that some ancestor of the Cruciferae had more carpels than are found in the 2-valved types of the present day, and that they were arranged in more than one whorl, how fit the symmetry of the 3-valved siliqua with one larger valve in the lateral plane on one side and a pair of smaller ones in the two opposing diagonal planes into the typical Crucifer ground-plan? Or how account for the absence in some of these extra-valved fruits of one of the fertile vascular cords supposed to represent the conjoined margins of contiguous valves, except by recognising that the sutural cord, with the surrounding tissue, forms by itself a whole carpel of a different type which here is completely suppressed? To look upon an abnormal organ as something *sui generis* is to credit the organism with the capacity to escape altogether from the bonds of inherited morphological restraint, a supposition which, surely, all our knowledge contradicts. But it is unnecessary, perhaps, to labour this point further, for the writer, having registered his protest, proceeds at once to discount it by acknowledging that the bulk of the evidence which I have brought forward in support of polymorphism does not come under this head.

The writer next arraigns me (p. 193) for not having produced any "clear comparative evidence" showing the evolution of a functional solid carpel. But does he, I would ask, withhold belief in all evolution where the actual intervening steps are not yet known? In attempting a new interpretation of data, in large part familiar, I aimed at building upon facts and avoiding, as far as might be, recourse to invention. But Mr Parkin appears to have strangely little interest in

¹ Very rarely such a fruit may occur at a higher point on the axis but only when it is evident that the conditions which obtain at the onset of the flowering period have again been reproduced, as e.g. after a check in growth.

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modern facts. He has acknowledged that his remarks are not the outcome of any close scrutiny of the submitted evidence. I have referred in my account to the case of *Triglochin* in which consolidation of a carpel can be seen in process of taking place, and have shown that in the Liliaceous series *Fritillaria*, Tulip, Lily, we can trace a very pretty series of stages in reduction along somewhat different lines. Other instances would almost certainly come to light as the enquiry widened. But if these examples sufficed to show that carpel polymorphism was a reality there was nothing to be gained at this stage by guessing, or by premature generalisation. But my critic is not satisfied. The examples cited relate to the consolidated carpel when sterile. He would know, here and now, what I conceive to be the evolutionary stages of this same type of carpel when it is fertile. After suggesting that I apparently regard compression (or shrinkage) as seen in *Triglochin* as the probable method by which consolidation is brought about, and pointing out that in this case it is difficult to conceive that such a carpel would retain its fertility, he allows it to be possible for me to extricate myself from this postulated difficulty, and proceeds to describe the stages by which such a carpel might be supposed to arise from a typical valve carpel in a gynoeceum with parietal placentation, incidentally, however, introducing the slight inaccuracy of placing the ovules in the final stage *on*, instead of *on either side of* the midrib. This description, which adds nothing to our knowledge, seems to assume that the syncarpous gynoeceum with parietal placentation is (or has been) of wide occurrence. But is this so certain? Indeed, is such a form of gynoeceum (in the accepted sense) a reality at all? I begin to doubt it. Already in the light of the polymorphic theory case after case of parietal placentation has gone by the board¹. Is it perhaps possible that the writer's hopeful suggestion may prove to amount to nothing more than that of deriving the solid carpel from itself? The line which the writer takes here and in his next point, and again in his discussion on the commissural stigma suggests that no array of facts which, looked at in the light of the polymorphic theory can at once be shown to fit together like the pieces of a jig-saw puzzle, but which, upon the orthodox interpretation, show no more connection than these same pieces when shuffled up together, will convince him if the phylogenetic picture cannot simultaneously be filled in.

¹ To the cases which have already been eliminated in the earlier accounts may now be added the Violaceae, Passifloraceae, Droseraceae, *Hypericum*, *Elodes* and *Melanthium* which is dealt with on p. 305 of the present account.

He has next to complain that he finds it difficult to grasp my views "as to the ancestral form of the Cruciferous gynoecium" (footnote 1, p. 194). Again I suspect that the root of the difficulty is that he has sought to discover in my account what is not there, *viz.* a complete phylogenetic scheme of the evolution of the gynoecium in this family. The very simple statement I ventured to make in this connection is that we appear to have *concrete* evidence here of the same tendency to reduction in carpel number which is held to be general throughout the Angiosperms. In more than a score of the genera in this family the reversionary 3- and 4-valved types of siliqua referred to above (p. 295) have been recorded. In *Tetrapoma* (*Nasturtium palustre*) even 5- and 6-valved fruits have been observed, indicating (on the polymorphic view) the presence of 10 and 12 carpels respectively. Thus whatever difficulty the writer may feel in imagining how such increased numbers of carpels are to be fitted into the Cruciferous ground-plan, the fact remains that these larger numbers actually occur. To hold (as the writer does) that the occurrence of numerous carpels is improbable because such a construction "conflicts strongly with the systematics [whatever they may be] of the family" seems to me no more logical than it would be to argue, supposing the genus *Megacarpaea* to be unknown, that the presence of numerous *stamens* is not conceivable in a Cruciferous type. For the construction of the androecium in the Cruciferae is a character of no less significance than that of the gynoecium. It is, indeed, of more, for it is unique. Yet *Megacarpaea* with its numerous *stamens* is a fact. Then why not a genus with numerous carpels? We have the counterpart of the typical Crucifer in the 2-valved *Chelidonium* among the Papaveraceae, why rule out the counterpart of such genera as *Meconopsis* or *Papaver* among the Cruciferae? But argument aside, a certain amount of evidence is already to hand indicating that the number of carpels present is generally likely to be higher in those genera which lack well-developed valve or pseudo-valve (semi-solid) carpels than in other genera in the same family in which these latter carpel types are present. It need not therefore surprise us if carpel numbers higher than those that have been recorded for *Tetrapoma* should occur in Cruciferous genera where none of the carpels form well-developed valves. At the same time I now think it probable that my original suggestion of a maximum ranging up to 40 or 50 is an over-estimate. This calculation was made on the supposition that in certain forms, which were not however obtainable for examination, small valve carpels alternated with the

solid members, but in allied species which have now been procured it has been found that there are no such intervening valves. There is also now no doubt that in the case of *Hypocotum* the carpel number was put too high. Preparations since made through the extreme base (stipe) of the ovary have shown that four of the longitudinal vascular cords which had been taken to be completely independent originate as lateral veins from the valve carpel midribs. This reduces the carpel number to that of the typical Crucifer, viz. 4. These corrections have been already incorporated in a further contribution on this subject now nearly completed, and as they in no way invalidate the general principles of the theory it seems unnecessary to enlarge further upon them here. Numerous carpels still remain an established fact, and in these circumstances the actual maximum is of no great moment.

4 From his remarks concerning *direction* of the vascular cords (p. 195) I gather that the writer has misunderstood the distinction which I drew between external features which are significant of carpel number and those which are not. External structures which are restricted to the course of vascular cords running a *separate* course from the base of the gynoeceum upwards (= cords of solid carpels) enable us to judge the carpel number from outward inspection. Structures occurring in connection with *lateral* (not therefore necessarily *horizontal* be it noted) veins as well as the midribs from which the veins arise obviously would not.

5 With regard to the commissural stigma the writer admits that the polymorphic theory affords a solution (p. 195), but having rejected the theory on other counts he must attempt some other explanation, and with the invention born of necessity he suggests that perhaps originally the stigmatic papillae covered the extent of the carpel apex, but that in the course of the phylogenetic history these papillae, for some reason, came to persist in some types over the midrib only and were suppressed on the apical margins, while in others they remained in this latter region and disappeared over the midrib. This mere paraphrased version of the facts, if I may so describe it, is no explanation; it advances us nothing and makes no attempt to relate stigma position with any other feature. As the writer would be willing to treat the Cruciferae separately from the great bulk of Angiosperms so he appears to be content to view the Crucifer stigma as an isolated and detached problem. Not to perceive that the varied configuration and disposition of the stigma have a real significance and furnish another link in a lengthening chain of

evidence would seem to show that with him the formula $G = 2$ for the Cruciferae has become an article of faith which not even such a stigma arrangement as we may see in *Cochlearia armoracia* (Fig. 1) can shake, although in this case a morphological fiction would have to be superadded to a phylogenetic myth in order to account for the quadri-fid form. To be driven to have recourse to such assumptions and at the same time to refer, as the writer does, to these stigma features as "awkward" facts which the polymorphic theory unable to explain must seek to get round, is such a topsy-turvy rendering of the position as to seem like wilful misrepresentation. Inasmuch as this theory provides, for the first time, a rational solution of the whole stigma problem which, on the orthodox view, has ever been, and remains, a contradiction. That division of labour should accompany diversity of form is not a source of difficulty but is, on the contrary, to be expected. But it does not necessarily follow that the distribution of this or that function will follow an invariable rule in relation to *one particular* morphological feature since other circumstances may affect these interrelations, such, for example, in the present case, as the relative heights reached by the solid and valve carpel midribs respectively. In the Stock the expanded valve and narrow solid carpels terminate at approximately the same level. The whole top of the valves are covered with stigmatic papillae. Whether the two stigmatic crests are actually continuous at their extremities is not easy to determine owing to the small dimensions of the intervening solid carpels in the early stages. But I am disposed to think that they may be, and that the solid carpels take a small part in completing the stigmatic ring. That both pairs of carpels can, and do function stigmatically is however clear in flowers of *Cochlearia armoracia* as shown in Fig. 1. But in some forms the valve carpel midribs fall far short of the top of the solid carpel cords and in such cases it is difficult to see how the valve members could furnish the necessary water supply to stigmatic papillae. It seems to me not improbable that in this diminished development of the valve carpel vascular system we may have a clue to the restriction of the stigmatic papillae to the solid carpels. This lack of a fixed interrelationship between stigma disposition and carpel type the writer considers to be "somewhat subversive of comparative morphology" (p. 196), but, as is apparent, this want of fixity in a particular function (not the only one, it is to be remembered) may result directly from variability in some other morphological character (other i.e. than the class of carpel). On this same ground the writer takes exception to

the notion of interplay between the carpel types, but such interplay is not a mere abstraction. I have already instanced the case of the earlier and later fruits of *Ceratocarpus* (*Corydalis*) *heterocarpa*. The same phenomenon can be seen in *Capsella Heegeri*. Many individuals having the lower fruits of the typical *Heegeri* pattern, i.e. rounded, the four carpels being nearly alike in shape, show a gradual transition in later flowers to the flattened *Bursa pastoris* form owing to lateral extension of the median pair of carpels and a corresponding diminution in the valves. The old bicarpellary scheme provides no clue to this class of facts and here, I suspect, even the writer's imagination fails him for he makes no reference to them.

In his remarks regarding the variable relation of the line of dehiscence to the line of junction of the carpels the writer falls into the same error as in his discussion on the variable position of the stigma. He leaves out of account the varying morphological factors which affect these relations. We may suppose that the line of dehiscence represents a line of relative weakness, and this line is often likely to coincide with the junctions of the carpels. Thus in the case of *Haematoxylon* among the Leguminosae dehiscence occurs down the middle of the two flat sides of the pod for in this plant the venation systems of the two carpels which are about equally developed do not, as in most of the genera, actually interconnect. Hence the line between the two systems might well represent the line of least resistance. Again, in *Medicago*, where one of the carpels has a well-developed system of horizontal lateral veins which extend right across the width of the pod, whereas the other has merely a midrib, the line of weakness will lie alongside this latter cord. In both these cases dehiscence takes place along the line of junction of the carpels. But in most of the Papilionatae horizontal lateral veins spring from both midribs and are continuous and firmly interconnected. Splitting of the pod is accomplished more easily by the drying up and rupture of the few ground tissue cells separating the twin bundles of each midrib than by the tearing across of the whole system of lateral veins from top to bottom. In another genus, *Carmichaelia*, both midribs become encased in sclerenchyma and form solid strands which cannot be split. The one or two lateral veins are negligible, being extremely tenuous; the fruit wall is exceedingly tough and leathery. The only possible region where the tissues can give way is at the boundaries of the sclerenchyma. It is here that dehiscence takes place, and the sides of the pod fall from the frame formed by the midribs like a pane from a window. In neither of these cases

does dehiscence follow the line of junction of the carpels but this fact is quite beside the question of the number and type of the carpels composing the ovary. So it may prove to be in the case of the silicula, for dehiscence resolves itself ultimately into a question of *tissue* distribution and interrelationships between incidence of strain and relative brittleness or toughness of cell membranes.

With regard to the application of the polymorphic theory to the Papaveraceae the writer has little to say beyond hazarding the conjecture that the numerous styles to be found in some early flowers of *Eschscholzia* might be able to be explained in some less intricate way than by supposing that they belong to numerous carpels (p. 198)! How this might conceivably come about we are not told. The implication that *Eschscholzia* alone has been studied is not perhaps intended. The suggestion, however, that the presence of numerous stigmas in some *Eschscholzia* flowers is a condition so peculiar as possibly to require some special explanation (the writer's favourite form of solution), which might be discoverable on further investigation, is altogether misleading. One may point out that one (so-called commissural) stigma to one solid carpel is almost universal throughout the family. The unique feature of *Eschscholzia* is not that it can, and under certain conditions does show numerous stigmas, but that it is in process of losing them. One further point is perhaps worth mention. After I had arrived at the conclusion that the *Eschscholzia* pistil is composed of 20 carpels I ventured to say that although an overwhelming majority of the flowers possess only 2 or 4 styles it was not unlikely that among the earliest flowers in the season some might be found with 20. Since making this statement I have come across a specimen with 19 styles or rudiments of styles.

Concerning the Resedaceae (see Figs. 2-4) it may be well to recall the position although it has been explained elsewhere. I examined members of this family at a very early stage in the enquiry before I had appreciated more than what the Cruciferae and Papaveraceae show regarding the characters of consolidated carpels. At the time the orthodox view appeared to offer the only possible interpretation of the otherwise (as it seemed) puzzling feature of the forking of the alternate vascular cords in *Reseda luteola*. But as soon as it had become clear through investigation of other families that dehiscence by a split in the middle line, though rarely found in the valve type of carpel is a common characteristic of the consolidated type, the case of *R. luteola*, in which it is to be noted that there is

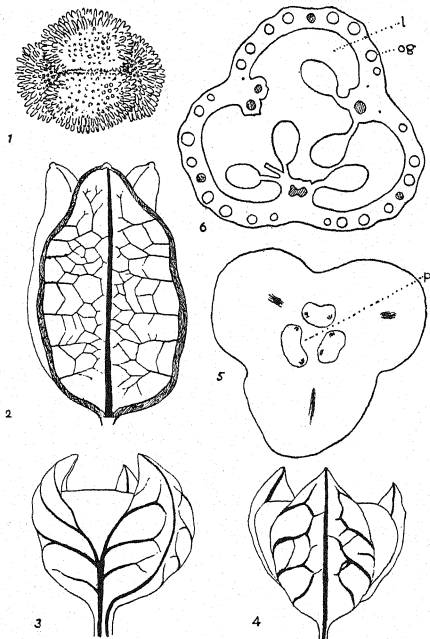


Fig. 1. *Cochlearia armoracia*. The stigma seen from above (highly magnified).

Fig. 2. *Reseda odorata*. The ovary seen from the back showing one of the three valves in surface view; in the centre the main vascular cord of a sterile carpel destitute of lateral veins; to right and left the main cord of a fertile carpel from which the secondary veins take their rise.

Figs. 3 and 4. *Reseda luteola*. Fig. 3. The ovary seen from behind and slightly to one side showing the forking cord of a fertile carpel and the secondary veins arising from it. Fig. 4. The same viewed from the front showing in the centre the cord of a sterile carpel with few inter-connections.

Figs. 5 and 6. *Hypericum Elodes*. Fig. 5. A transverse section of the ovary taken below the level of formation of the loculus showing the midribs of the three outer sterile carpels cut obliquely, and nearer the centre the three large vascular cords with twin xylem bundles belonging to the three fertile carpels. Fig. 6. The same taken from a higher level. The ovary has now become unilocular. The ovary wall shows the six vascular cords and a number of oil glands. *l*, loculus; *og*, oil gland; *p*, pith.

the further puzzle that the orientation of the gynoeceium is the reverse of that in *R. odorata*, was at once made plain. Evidently the three fertile cords did not represent the meeting edges of three valve carpels but the midribs of three additional consolidated members, and this revised interpretation is given in my paper on Carpel Polymorphism¹. The writer admits in a footnote (p. 199) that he overlooked this correction when writing his remarks (which he nevertheless has allowed to stand) and that he realises that they may not now apply. I may therefore pass them by, merely calling to mind that the interpretation of the ovary of *R. luteola* and *R. odorata* on a basis of 6 carpels instead of 3 explains, as I have already shown, not only the difference in orientation in the two species but the presence of stigmatic papillae in some strains of the last-named species over each of the 6 vascular cords. On the orthodox view that $G=3$ it is exceedingly difficult to account for 6 stigma points equidistant from one another.

The gist of the writer's comments on my interpretation of certain features in the Leguminosae and Liliaceae is that they do not harmonise with the views which he holds regarding the phylogeny of these families. It comes then to this. We have on the one hand a body of observed facts and an interpretation which covers those facts and links them intelligibly together. On the other a total disregard of this evidence which the writer does not even trouble to examine though many of the facts clearly call for some explanation if the polymorphic theory is rejected; and phylogenetic argument which the writer applies as though a phylogenetic scheme were a criterion of evidence instead of a chronicle compiled from and designed to embrace all the facts, but inevitably imperfect, and tentative at the best. As regards the particular case of *Melanthium virginicum* L. I must at once acknowledge that I no longer stand by my earlier, too ready acquiescence in the orthodox account². At the time the only material available for investigation was a single herbarium specimen of a dehiscent fruit. In the already split vascular cords I failed to appreciate the oneness of origin of the two halves and in the absence of any evidence pointing to the contrary I accepted too unquestioningly the traditional standpoint. But after the case of *Reseda luteola* had been cleared up, and when later it was found that in *Hypericum Elodes*, in some ways a comparable case to *Melanthium virginicum* (see Figs. 5 and 6), the position of the

¹ *Ann. Bot.* 39, p. 129.

² See "Carpel Polymorphism I," *loc. cit.* p. 166.

placentae on the wall of the ovary did not indicate, as would at first sight seem to be the case, a different mode of placentation from that occurring in the plurilocular species of the genus, I more than suspected that the division of the upper part of the *Melanthium* ovary into 3 closed follicle-like structures, and the appearance of the 3 valves of the ripe fruit had proved deceptive, but no satisfactory material was then to hand. The appearance of Mr Parkin's article at a moment when it was possible to procure fresh material has led me to re-investigate the point and now affords me opportunity to retract my earlier statement. A transverse section through the base of an undehiscent ovary shows clearly the 3 centrally-placed cords of 3 consolidated carpels in addition to the 3 outer cords in line with the loculi. There prove to be, in fact, 6 carpels here as in other members of the Liliaceae. The distinctive feature of *Melanthium* consists, it is now plain, not in possessing only 3 carpels as compared with 6 in the other genera but in having, as can easily be seen in undried material, so much ground tissue between the twin bundles of the fertile cords that splitting in the midline of the fertile carpels comes about more easily than a rupture in the sterile members whose midribs are formed of single bundles¹. In the words of the older terminology, now, however, carrying a somewhat altered meaning, dehiscence is septicidal, not loculicidal. It may be well, however, this correction notwithstanding, to expose the unsoundness of the writer's argument. The combination of carpel types in Liliaceae appears generally to be 3 solid, outer, sterile with 3 inner, semi-solid and fertile. Had *Melanthium virginicum* actually possessed 3 episepalous valve carpels this fact would not have afforded reason to expect to find here, if anywhere, in this family signs of the evolution of the solid carpel—would not, in fact, have constituted a "weakening admission." On the contrary the presence of an episepalous whorl of *expanded* valve carpels (were it a reality) would be the very reason why such an occurrence might be considered highly improbable.

On the last page of the article the writer leaves phylogenetic argument and deigns to turn to facts but all he has to offer by way of their explanation are vague suppositions of a purely Lamarckian character illustrated by a single example taken from my account, but unfortunately resting upon a misconception. Of the value of these explanations, evidently devised *ad hoc*, I leave the reader to form his own judgment. The writer then concludes with an apologia in which he mentions that he has found the subject "by no means easy

¹ This is illustrated by figures in the forthcoming account.

to grasp." One may be pardoned perhaps for suggesting that part of the difficulty may not be inherent either in the theory itself or in the mode of presentation but may arise from the endeavour to fit a new outlook into a preconceived scheme.

Although not directly called for by Mr Parkin's criticisms a few general remarks in regard to the different lines of evidence brought forward in support of the theory of carpel polymorphism may not be out of place. Upon the value of evidence derived from abnormal structures I have dwelt already and need say no more. The ontogenetic evidence at our disposal is somewhat scanty and cannot safely be relied upon as sole guide as the following example will show. The androecium in the Fumariaceae, according to Payer, appears in the first instance as two protuberances, one on each side of the flower. Each protuberance develops into a central whole stamen and a half stamen on each side. Yet, although each such group is derived from a single protuberance, does any one doubt that the three structures represent what they appear to be, and that each protuberance is equivalent to $\frac{1}{2}$ + $\frac{1}{2}$ stamens, the halves arising through congenital splitting of the median pair? It is not a little ironical that the ontogenetic evidence in the case of the Crucifer gynoeceium should plainly point, on Eichler's own showing¹, to the presence of 4 carpels, and that nevertheless, in order that the appearances might be brought into line (as he supposed) with other facts, they should have been interpreted by him as denoting only 2, and have been so accepted by almost every other systematist. But by far the most fruitful line of investigation has undoubtedly been the study of the course of the vascular cords, a method which has proved in the past of the greatest value in enabling us to arrive at a true comprehension of the real nature of many floral structures, of which the androecium of the Orchidaceae—to take but a single example—is an excellent case in point.

The accompanying figures have been drawn by Miss D. F. M. Pertz to whom I here tender my grateful thanks.

¹ See *Flora*, 1865, Pl. VI, fig. 13 and p. 555.

REVIEW

The Classification of Flowering Plants, by ALFRED BARTON RENDLE, M.A., D.Sc., F.R.S., P.L.S., Keeper of the Department of Botany, British Museum. Vol. II. Dicotyledons. Cambridge University Press. Price 30s.

Since the announcement of a new book on the Classification of the Dicotyledons arouses a pleasant sense of anticipation in the breast of the student of systematic botany, one opens the second volume of Dr Rendle's *Classification of Flowering Plants* with eager curiosity. But a feeling of disappointment is soon experienced. In his preface Dr Rendle writes, "No apology can be adequate for a delay of more than twenty years in the appearance of the second volume of this work." It is unfortunate, however, that since the work was planned, and the bulk of it written, our views on some aspects of the subject have altered considerably. The description of each family seems to resemble that in any of the existing standard books on the subject; in fact one begins to search for anything, either in statement of fact or in treatment, that is new.

The enlarged floral diagrams after Eichler are very clear; but has there really been so little advance in our knowledge of floral structure in the half century since they were constructed that they should reappear today without discussion or even brief reference to the difficulties which they do not solve? In the *Blüthendiagramme*, in which these diagrams originally appeared, attention is drawn to those morphological features which are at variance with the formal scheme they depict; but here the reader, who is very likely unacquainted with Eichler's work, detects no suggestion that there are many morphological difficulties which these diagrams do not meet. This omission leads one to ask for whom the book is intended. Is its purpose merely that of description to serve the needs of the pure systematist? If that is the author's primary object, this cut and dried information may adequately serve his end. But in a work of this scope one looks for more; in fact such presentation of the matter as will show in what direction further research needs to be undertaken. The bald statement of morphological incompatibilities without comment, either gives too little to the already informed, or asks too much from the unenlightened reader.

Let us cite a few examples of what is meant. On p. 152 the two-valved capsule of *Epimedium* is derived from a single carpel. The evidence that the capsule of *Epimedium* consists of two carpels seems remarkably clear. It possesses two systems of venation, which do not coalesce, and it is difficult to doubt Miss Saunders's conclusion (*Annals of Botany*, Jan. 1925, p. 131) that the capsule consists of one fertile semi-solid carpel bearing a style and stigma, and one sterile valve-like carpel with primate venation.

On p. 45 in regard to the Ulmaceae it is said that "the abortion of the hinder carpel does not extend to the style," and on p. 51 of the Urticaceae, "in *Morus*—its (the hinder carpel's) presence is indicated only by a style similar to that of the anterior carpel." From these last two statements the student is presumably to infer (1) that the presence of a loculus is the criterion of the presence of a carpel, and (2) that in the absence of a loculus it is possible for the upper portion (style) of a carpel to be present without the basal portion of which it is the termination.

On p. 270 we read: "No satisfactory explanation of the obdiplostemony in *Geranium* has been given." Is this quite fair to the student? Is not Miss Saunders's explanation of obdiplostemony eminently satisfactory? Must it not be taught to students until someone suggests a still more satisfactory explanation?

On p. 279: "False septa divide each carpel into two chambers" (Linaceae). Again should one be content with the term "false" septa, and with simply leaving it at that, when an explanation has been suggested which does away with the "falseness."

The description of the Willows is likely to cause students great difficulty. On p. 7 it is stated that the stamens in *Salix incana* and *S. purpurea* are "united in a ring at the base." Is this true? On p. 10 we read: "*Salix*...may be divided into three tribes, characterised by the number or cohesion of the stamens: (1) *Pleiandrae*: stamens free and three or more in number, including the British species *S. triandra*, *S. pentandra*, *S. fragilis* and *S. alba*." As the two last-mentioned species have never more than two stamens, surely this statement calls for some explanation.

On the Continent during recent years much work has been done on sero-diagnostic methods of investigating plant affinities. When such illustrious systematists as Engler and Wettstein take this method of research very seriously it seems strange that the author of the work under review should make no mention of it at all.

With all these defects, many of which doubtless spring from the lamentable delay in its appearance, the book contains a mass of information arranged in a way which will be of great use to students. The sequence, with rather numerous exceptions, is that of Engler's Syllabus. The book is well printed, and contains a wealth of good, clear, illustrations.

H. G.-C.

THE NEW PHYTOLOGIST

VOL. XXV, No. 5

24 DECEMBER, 1926

STUDIES ON THE HETEROKONTAE

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(With 13 sets of figures in the text)

A FULLER account of the five members of the Heterokontae which form the subject of the present study is given in a work entitled *Étude sur les Hétérokontes* published by the author in Geneva, Sept. 1925¹. The structure and life-histories of these algae were studied in the botanical laboratories of the University of Geneva with the invaluable counsel and guidance of Professor R. Chodat, who suggested that an abridgement of the original work be published in English.

The group of the Heterokontae, which comprises about twenty-two genera, is an exceedingly interesting and important one. A number of valuable observations have already been made on certain genera. Until, however, the structure and life-history of each member have been thoroughly investigated, we cannot hope for a satisfactory classification of the group. As our knowledge of each genus increases, a fuller understanding of the limits of the group and its systematic position will ensue.

Representatives of five genera were studied:

- (i) *Chlorobotrys stellata* Chodat.
- (ii) *Botrydiopsis minor* Chodat.
- (iii) *Characiopsis ovalis* Chodat.
- (iv) *Heterococcus viridis* (Gern.) Chodat.
- (v) *Tribonema bombycinum* Derb. et Sol. (*Conferva bombycina* Ag. var. *intermedia*).

¹ Thesis presented to the University of Geneva for the degree of D. ÈS SC. Thèse no. 777, Genève, Imprimerie Jent. Société Anonyme, 9-11 Rue Necker. 1925.

(i) *Chlorobotrys stellata* Chodat

Chlorobotrys stellata is a small unicellular green or yellow-green alga which has a firm strong outer wall containing a certain amount of silica. The specific name "stellata" was chosen by Professor Chodat on account of the characteristic stellate or hexagonal form assumed by the asexual spores under certain conditions (Fig. I, 1, 3, 11). The mature cells are normally spherical and attain a diameter of 11-12 μ . The cells may exist singly, but very often they occur in pairs or in masses which are held together by a colourless jelly-like membrane consisting largely of pectic substances (Fig. II, 22, 24). As many as thirty cells may be thus held together forming an irregular aggregation of cells. There is not the slightest tendency to regularity of arrangement in these "colonies." Under certain conditions such colonies are practically absent, also stellate walls. This condition appears to be associated with a lessened production of pectin in the cell-walls, and is evidently a variable feature.

A single cell may be furnished with one large parietal chloroplast (Fig. I, 6). This may later fragment into several (four or more) separate chloroplasts (Fig. I, 3). Xanthophyll is present in addition to the chlorophyll, and the product of assimilation is oil, never starch as in the Chlorophyceae. The oil appears as minute granules, or as a large somewhat angular body (Fig. I, 13). A reddish spot, probably consisting of carotin, is often though not invariably present in the cell. Each cell is provided with a single, rather small nucleus (Fig. I, 17, 18).

Asexual reproduction takes place by means of vegetative division, the formation of motionless asexual spores, also by zoospores. The asexual spores are usually formed in pairs within a mother cell (Fig. I, 7), and the old wall eventually becomes gelatinous, and often persists for some time, holding together the daughter cells. By the repetition of this process the irregular "colony" is produced. Both asexual spores and daughter cells formed by vegetative division may show the characteristic stellate form under suitable conditions. When growth in size takes place, however, this form is gradually lost and the cells assume the spherical character of ordinary mature individuals.

Zoospores are formed, usually three or four in each zoosporangium (Fig. II, 27, 28). These are somewhat pear-shaped, with a colourless anterior end from which spring a pair of unequal cilia (Fig. II, 29-32). A single chloroplast is present, and a red spot is sometimes visible at the anterior end. Movement is very active.

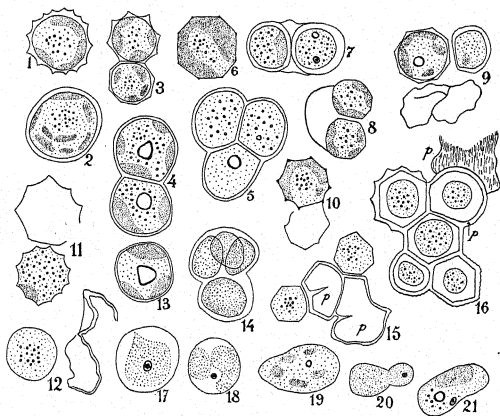


Fig. 1. *Chlorobotrys stellata* Chodat.

1. Asexual spore with stellate wall.
2. Vegetative cell with smooth wall showing numerous chloroplasts and small oil drops.
3. Vegetative cell-division. The cells are assuming the stellate character before they have separated.
4. Vegetative cell-division. The cells have smooth walls and contain a large oily body, also small oil drops.
5. Vegetative cell-division—a group of three cells being formed.
6. Polyhedral cell, appearing octagonal in optical section, the angles being thickened. One large hollow chloroplast is seen.
7. Formation of asexual spores within a mother cell, whose wall persists.
8. Two asexual spores assuming the polyhedral form, while still connected by the pectic wall of the mother cell.
9. Two spores entirely liberated, the pectic wall which originally enclosed them lying near.
- 10-12. Stellate cells rejecting the primary membrane. The stellate form is retained in 10 and 11.
13. Cell containing large angular oily body.
14. Vegetative reproduction, four daughter cells being formed within a mother cell.
- 15, 16. Persistent pectic membranes (*p*) binding the cells together.
- 17, 18. Cells stained with haematoxylin showing nuclei.
- 19-21. Irregular forms.

All $\times 1650$ except 17 and 18, which are $\times 1800$.

No conjugation of these motile cells was ever observed. The zoospores, after a short period of active movement, come to rest, and gradually assume the characters of ordinary vegetative cells.

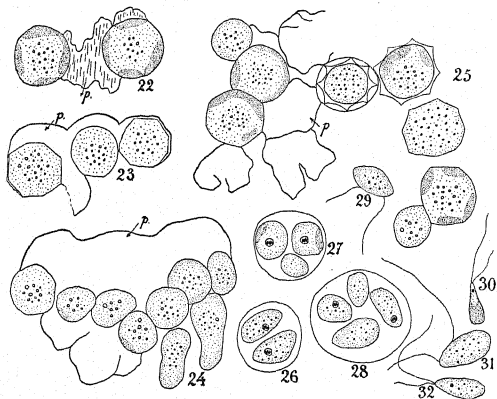


Fig. II. *Chlorobotrys stellata* Chodat.

22-25. Cells bound together by pectic membranes to form irregular "false coenobia."

26-28. Zoosporangia containing zoospores.

29-32. Zoospores with two unequal cilia.

All $\times 1656$.

(ii) *Botrydiopsis minor* Chodat

Botrydiopsis minor is a small unicellular alga which exhibits considerable uniformity in general appearance (Fig. III, 1, 2). The cells are commonly globular, but are sometimes slightly oval or elliptical in shape. Aggregations of cells are occasionally seen, but filaments have never been observed. Mature cells are usually about 9μ in diameter, but at times larger cells (12μ) may be found.

Each cell is provided with a firm colourless cell-wall which usually contains much pectin. No silica appears to be present, but there is often, though not invariably, a certain amount of cellulose. Growth of the young cell takes place by the rejection of the primary membrane (Fig. III, 4), as in *Chlorobotrys stellata*. A curious feature of

the cell-wall is the tendency to form one or more thickenings, which may be very slight, or sometimes very pronounced (Fig. III, 12-14). Large numbers of cells, however, are devoid of these thickenings.

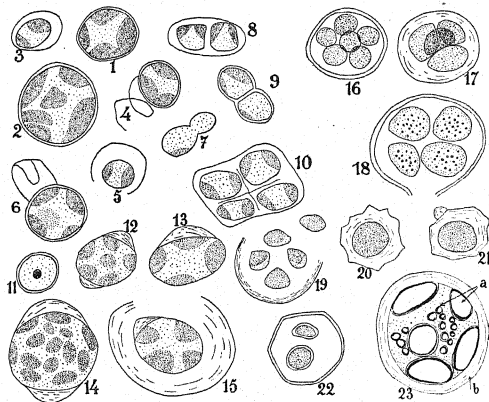


Fig. III. *Botrydiopsis minor* Chodat.

1. Young vegetative cell.
2. Older cell with numerous discoidal chloroplasts.
- 3-6. Cells whose primary membranes are being rejected.
7. Cell showing "budding."
- 8-10. Cells undergoing vegetative multiplication.
11. Cell stained with safranin showing single central nucleus.
- 12-15. Cells showing side thickenings of the wall. In 15 the thickening has taken place while the cell is still surrounded by the thick pectic covering of a mother cell.
- 16-19. Formation of autospores in autosporangia. In 17 a tetrad is seen. 18 shows the rupture of the pectic wall. In 19 the autospores are seen escaping.
- 20-22. Cells with thick pectic wall which shows a distinctly stellate or hexagonal character. 21 shows a projection of the pectic wall.
23. Cyst from the red margin of a culture, with large oil drops (a), and thick pectic wall (b).

All $\times 1650$.

A number of flat discoidal chloroplasts are usually observed in each cell, the number varying from two to eight, or even more. Xanthophyll is present, and oil, never starch, is formed as reserve food. No red spot is visible.

Asexual reproduction takes place very freely by the formation of autospores, a considerable number being formed in each autosporangium (Fig. III, 16-19). The wall of the latter becomes very thick and pectic, and ultimately the autospores escape. They grow without rest and quickly assume the vegetative characters of an ordinary cell.

Zoospores are formed by the division of a mother cell. The division is quite irregular. Twelve to eighteen zoospores are commonly formed in each zoosporangium (Fig. IV, 24-27).

After swarming in the mother cell for some minutes the zoospores escape one by one, the enclosing wall having become gelatinised. Each zoospore has two cilia which are slightly uneven in length, and stand out stiffly from the anterior end (Fig. IV, 33-35). A single chloroplast is present, which is often folded giving the appearance of two. A number of colourless granules are often seen, but no pigment spot was ever observed. The dimensions of the zoospores are: longitude 8μ , latitude 3μ .

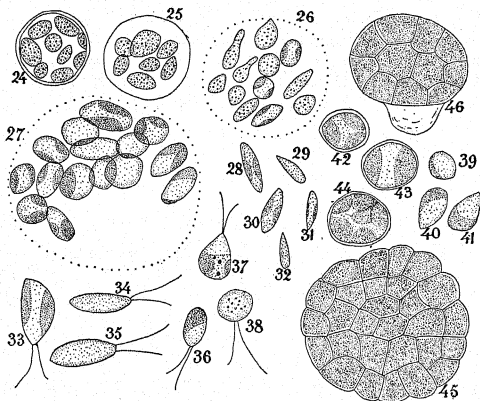
Smaller zoospores (longitude $4-5\mu$, latitude 3μ) were also observed (Fig. IV, 36-38). These are formed in the manner described above, and are invariably provided with two unequal cilia, and in all respects are similar to the larger forms.

No conjugation of these motile elements was ever observed, though it is claimed by Borzi⁽⁷⁾ for *B. arhiza* that conjugation occurs, and he figures gametes having cilia of equal length and provided with a red spot. He also describes the zoospores as having one cilium only. It appears probable, therefore, that Borzi has made an error of observation, and it is likely that the so-called "gametes" are cells of another alga—possibly *Chlamydomonas*, which had gained access to his cultures.

In old cultures of *B. minor* on media containing sugar a large number of cysts are formed, each having a thick wall and containing a large quantity of oil and carotin (Fig. III, 23). It is the presence of the latter that gives the brick-red appearance to the cells when seen in mass. If transferred to a fresh medium these red cells quickly divide and give rise to ordinary green cells.

(iii) *Characiopsis ovalis* Chodat (*Monodus ovalis* Chodat)

Characiopsis ovalis is an epiphytic alga which is commonly attached to freshwater filamentous algae or other aquatic organisms. In form it bears a pronounced resemblance to certain species of *Characium*, with which the genus has often been confused hitherto.

Fig. IV. *Botrydiopsis minor* Chodat.

- 24-27. Zoosporangia containing zoospores. [All four represent the same zoosporangium at different stages of development.]
 24. Zoospores are motionless at this stage.
 25. The wall of the zoosporangium is thinner but is still distinctly visible. The zoospores are beginning to swarm and change their shape as they move.
 26. The zoosporangium has increased in size and the wall is now practically invisible. The zoospores are swarming actively.
 27. The zoosporangium has greatly increased in size—the wall is now quite invisible. Swarming continues.
 28-32. In this condition the zoospores escaped, one by one.
 33-38. Fully developed zoospores with two unequal cilia.
 39-41. Zoospores which have come to rest, and have lost their cilia. Each is provided with a single chloroplast.
 42-44. Stages in the germination of the zoospore showing increase in the number of chloroplasts.
 45. Zoosporangium from which the zoospores have failed to escape, and are forming a spherical mass of cells.
 46. A mass of cells similar to 45 but provided with a side thickening of the outer wall similar to those of 12-15.

All $\times 1650$.

There are, however, marked differences between the two genera. *Characiopsis* possesses marked Heterokontean characters which separate it at once from *Characium*, a typical member of the Chlorophyceae.

Characiopsis ovalis is unicellular, and the cells usually occur singly, though small groups are occasionally seen adhering together

(Figs. V, VI). Each cell is commonly provided with a delicate stalk of variable length. In some cases this is extremely short or practically absent. When present, the stalk ends in a small flattened disc which is seen with some difficulty (Fig. V, 8). A beak-like projection may occur at the opposite end of the cell (Fig. V, 10, 27, etc.).

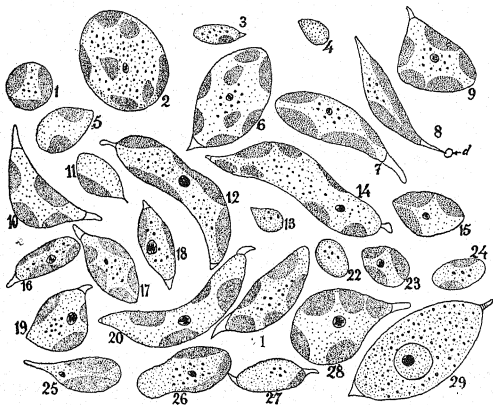


Fig. V. *Characiopsis ovalis* Chodat.

1-29. Cells assuming different forms. A stalk is present in some of the cells, e.g. 3, 6, 7, 9-12, 14, 16, 21, 25, 27, 29. In 8 is shown a small disc of attachment. In 10, 12, 14, 18, 20, 27, 29 a beak is present in addition at the opposite pole of the cell. Most of the cells contain numerous discoidal chloroplasts and in addition a red spot, more or less centrally placed, is present in some examples (2, 6, 7, 9, etc.). 29 shows the red pigment spot within a large vacuole. The cytoplasm of many of the cells is seen to contain granules of oily material, e.g. 2, 3, etc.

All $\times 1650$.

The form of this alga varies exceedingly. Indeed, the great variability of its form is one of its most characteristic features. In any pure culture a large number of different forms is usually seen. Fig. V shows a number of cells taken almost at random. The shape may be oval, elliptical, lanceolate, pear-shaped, spindle-shaped, etc. Not only do the cells differ in form, but variation also occurs in respect of (a) the presence or absence of a beak at the apex, (b) the

presence or absence of a stalk and, if present, its relative length, (c) the size of the cells, the limits being roughly: longitude 4.5μ – 24μ , excluding stalk and beak (the commonest being about 12μ); latitude 3μ – 9μ . The variability is much less pronounced in floating cells of

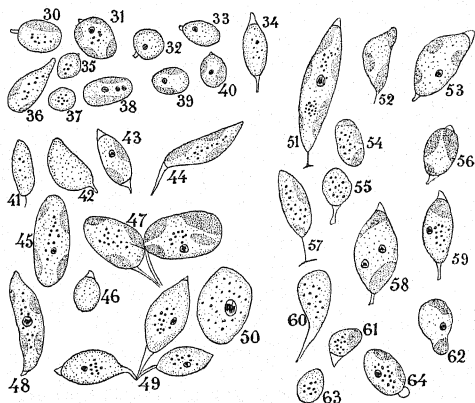


Fig. VI. *Characiopsis ovalis* Chodat.

30–40. A group of floating cells taken from a liquid culture near the bottom of the flask. The cells are almost without exception of the simpler type—with or without a stalk, or beak. Very occasionally a cell of the type shown in 34 is seen.

41–50. A group of cells taken from the bottom surface of a glass vessel containing a liquid culture. The cells show a far greater variety of form. A stalk is often present, also a beak at the opposite pole of the cell. 49 shows a group of three cells adhering by their stalks.

51–64. A typical group of cells from the sides of a glass vessel containing a liquid culture. A great variety in the form and size of the cells is again seen. Stalks and beaks are often present.

All $\times 1650$.

a liquid culture than those which are attached to the walls of the containing vessel where a great degree of variability occurs (Fig. VI).

The cell-wall of *Ch. ovalis* is rather delicate and is composed of cellulose, though in many cases pectic substances are present also. The stalk and disc, also the beak when present, are invariably of the same nature as the cell-wall.

Each cell contains a number of disc-shaped yellow-green chromatophores, which vary from two to eight or ten in number or even more. Xanthophyll is present, and small granules of an oily nature are usually present in the cell. A large red spot probably composed of carotin is frequently seen.

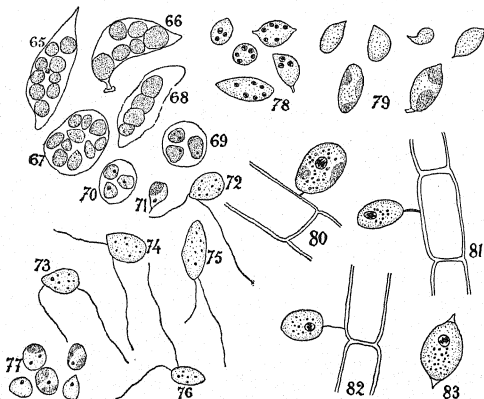


Fig. VII. *Characiopsis ovalis* Chodat.

65-70. Vegetative cells which have become zoosporangia containing a varying number of zoospores. 68 shows the dissolution of the wall prior to the liberation of the zoospores.

71-76. Zoospores. Two unequal cilia are seen in 72-76.

77. A group of zoospores which have come to rest, forming young cells.

78. A group of cells stained with safranin showing numerous nuclei in each cell.

79. Young cells showing the early establishment of one or two poles of the cell.

80-82. Cells of *Ch. ovalis* becoming attached to threads of *Tribonema bombycinum*.

83. Cell showing the formation of the stalk and beak from a simpler form.

All $\times 1650$ except 78, which is $\times 1800$.

The cells of *Ch. ovalis* differ from the other members of the Heterokontae studied in the possession of several nuclei in the cell. From three to six were usually found (Fig. VII, 78).

The reproduction of this alga is commonly effected by zoospores. No form of vegetative reproduction or formation of autospores has been observed. An ordinary vegetative cell becomes a zoosporangium

by the division of the contents into a number of small rounded bodies, about three to ten being formed in each cell (Fig. VII, 65-70). No regularity is observable in the division. The zoospores escape on the dissolution of the wall and move about rapidly. Each contains one chloroplast and a red pigment spot is in some cases present. Two unequal cilia spring from one end (Fig. VII, 71-76). The shape of the zoospore constantly changes as it moves. After a time movement ceases, the cilia are lost and the two poles of the cell are established very early.

No conjugation of the motile cells has been observed as Borzi (11) describes. He figures the zoospores as having one cilium only, and also describes the conjugation of isogametes to form a zygospore. As these observations have never been confirmed, however, they should be accepted with reserve.

The genus *Characiopsis* has hitherto been made to include a number of species¹, many of which have been based on differences in size, form, presence or absence of a stalk, number of chloroplasts, etc., all of which have been shown to be variable characters in *Ch. ovalis*. Such characters are therefore not reliable as a basis of classification. The examination of each supposed species under conditions of pure culture over a considerable period is the only way of arriving at a clear idea of the limits of the different species.

(iv) *Heterococcus viridis* (Gern.) Chodat

Heterococcus viridis is a green or yellow-green alga which occurs in water or in damp situations such as on the bark of trees and fences. The cells may occur singly when their form is usually spherical (Fig. VIII, 1, 5). They exhibit, however, considerable variation in shape and may be elongated, elliptical, broadly pear-shaped, slightly crescentic, etc. The largest of the spherical forms commonly attain a diameter of 12μ although larger cells have occasionally been observed.

Heterococcus viridis exhibits a high degree of polymorphism. In many cultures filamentous forms are met with in addition to the forms described above. The filaments are usually very short and consist of about two to eight cells (Fig. VIII, 3, 6, 11; Fig. IX, 18). They may be simple or branched and the cells composing them may be cubical or sometimes barrel-shaped. Flat plates of cells are also found, consisting of small colonies of two to many cells which show

¹ Lemmermann (14) gives eighteen species. Pascher (15) more recently (1925) enumerates twenty-seven species.

no regularity of arrangement (Fig. VIII, 7). Many of these forms show a marked likeness to the alga described as *Protococcus vulgaris*¹. As these algae thrive in similar situations it was inevitable that in the past they should have often been confused. They are, however, quite distinct, as the present study will show.

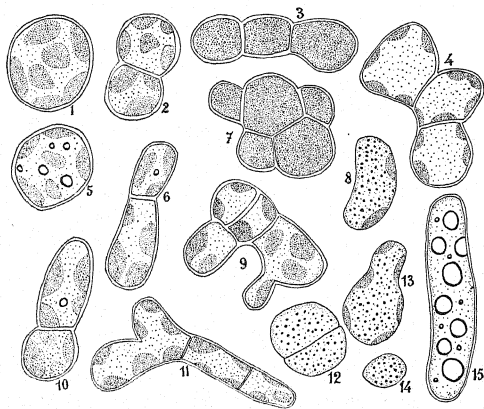


Fig. VIII. *Heterococcus viridis* Chodat.

1-15. Various forms, unicellular and multicellular, assumed by *Heterococcus viridis*.

1, 5, 8, 13-15. Unicellular forms. 1 shows numerous chloroplasts. 5 and 15 show large oil drops. In 8, 13, 14 the cells are filled with small oil drops.

7, 9. Irregular plates of cells formed by vegetative divisions.

3, 4, 6, 10, 11. Simple filamentous forms.

In 3, 7, 8, 12 and 14 no separate chloroplasts are visible, and the cells are uniformly green and packed with minute oil globules.

All $\times 1650$.

Each cell of *Heterococcus viridis* is invested with a fairly thick wall which contains a considerable quantity of pectic substances, although some cellulose is often present. The number of chromatophores is variable. In young cells there may be one or two only—later there may be as many as twenty or thirty in each cell. They are yellow-green owing to the presence of xanthophyll and are discoidal

¹ As, for example, that figured by West (62), Fig. 118.

in shape. Small oily granules or refractive drops are usually present (Fig. VIII, 5, 15), but no trace of starch has been found. Each cell contains a single nucleus (Fig. IX, 19, 20), but a red spot is completely absent.

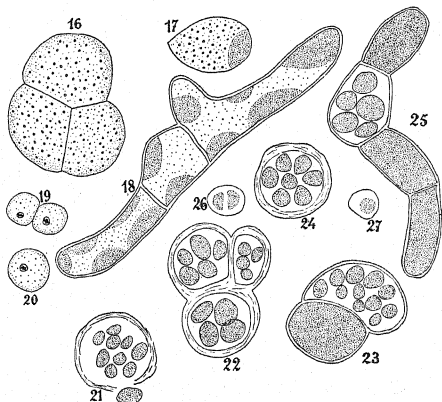


Fig. IX. *Heterococcus viridis* Chodat.

16-18. Further forms, unicellular and multicellular. In 16 no separate chloroplasts are visible, and the cells are crowded with minute oil granules.

19, 20. Cells stained with haematoxylin showing nuclei.

21-25. Autosporangia containing numerous autospores. 21, 22 and 24 show the thick pectic wall of the autosporangium, which in 21 has become ruptured, and the autospores are being liberated. 25 shows one of the vegetative cells of a filamentous form which has become an autosporangium.

26, 27. Showing liberated autospores which are beginning to germinate. 27 contains one plate-like chloroplast which does not completely fill the cell. In 26 this has divided to form two chloroplasts.

All $\times 1650$.

Vegetative multiplication takes place very freely. Asexual spores, both motionless and motile, are also formed. Non-motile spores (autospores) are produced by repeated division of the contents of a cell (Fig. IX, 21-25). The number is variable, and no regularity in their formation is observed. As the spores ripen the wall of the mother cell becomes highly pectic and ultimately decays, or the spores may escape by gaps in the cell-wall.

Macro- and micro-zoospores are formed in zoosporangia. The macro-zoosporangium (Fig. X, 28-30) when ripe contains ten to twenty macro-zoospores. The wall gradually dissolves while the zoospores are swarming inside, finally they escape one by one. Their dimensions are: longitude 6.7μ , latitude $1.8-2\mu$. They are provided with two unequal cilia and one chloroplast (Fig. X, 31-33). After

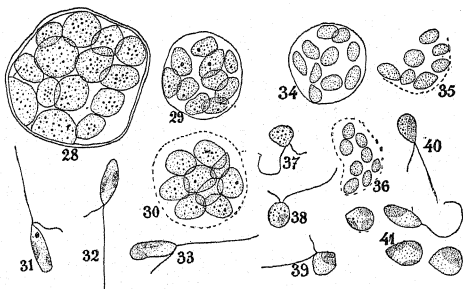


Fig. X. *Heterococcus viridis* Chodat.

- 28-30. Macro-zoosporangia containing macro-zoospores. In 29 the zoospores were actively moving and the wall was distinctly visible. In 30 the wall of the zoosporangium has become almost invisible.
- 31-33. Macro-zoospores, each with a single folded chloroplast, and two unequal cilia.
- 34-36. Micro-zoosporangia containing seven micro-zoospores. 35 and 36 show the gradual dissolution of the wall of the zoosporangium. The micro-zoospores escape slowly, one by one.
- 37-40. Micro-zoospores each provided with two unequal cilia. 38-40 show a single chloroplast.
41. A group of zoospores which have come to rest, showing increase in the number of chloroplasts. The longer cilium is still attached to one of the zoospores.

All $\times 1650$.

a time the zoospore comes to rest, the chloroplast divides into two and the spherical form is assumed (Fig. X, 41).

The micro-zoospores (Fig. X, 34-40) are formed similarly and have a similar structure except that they are nearly spherical. The diameter is $2.7-3\mu$. They differ, however, in the extreme sluggishness of their movement both while within the micro-zoosporangium and after their escape. A pair of unequal cilia become visible after their escape. The movement is a slow gliding movement in marked contrast with the rapid jerky movement of most zoospores.

No conjugation was observed, the zoospores of both types coming to rest after a period of movement, and forming ordinary individuals.

(v) *Tribonema bombycinum* Derb. et Sol.

(*Conferva bombycina* Ag. var. *intermedia*)

This well-known filamentous alga with its barrel-shaped or cylindrical cells agrees with the genera already described in its

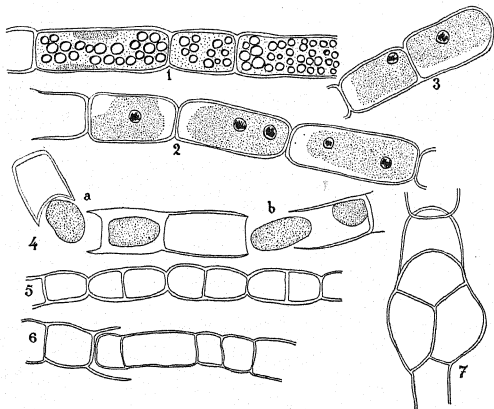


Fig. XI. *Tribonema bombycinum* Derb. et Sol.

1. Filament showing vegetative cells crowded with granules of reserve material. Individual chloroplasts almost invisible.
- 2, 3. Cells stained with haematoxylin showing nuclei. The longer cells of the filaments contain two nuclei, while the shorter ones contain only one.
4. Formation of aplanospores. Filament separating to liberate aplanospores. Usually one aplanospore only is formed in each cell as in *a*. Occasionally two are formed in the same cell as in *b*.
- 5, 6, 7. Formation of akinetes. In 5 and 6 the akinetes form a linear series. In 7 the cell division has taken place in more than one direction.

1-4 $\times 1650$. 5 $\times 750$. 6, 7 $\times 1200$.

possession of Heterokontean characters such as the form of the chloroplasts, the presence of xanthophyll and the oily reserve product. The latter often appears as fairly large opaque whitish granules which are sometimes very numerous (Fig. XI, 1). Two nuclei are often seen in the cell (Fig. XI, 2).

There are three methods of asexual spore formation, viz. by aplanospores, akinetes and zoospores. Aplanospores are formed singly or occasionally in pairs within a mother cell. They are set free by the dissociation of the filament into H-shaped pieces (Fig. XI, 4; Fig. XII, 14).

Akinetes are very frequent and usually occur in a chain-like series (Fig. XI, 5, 6) or less commonly in a small group (Fig. XI, 7).

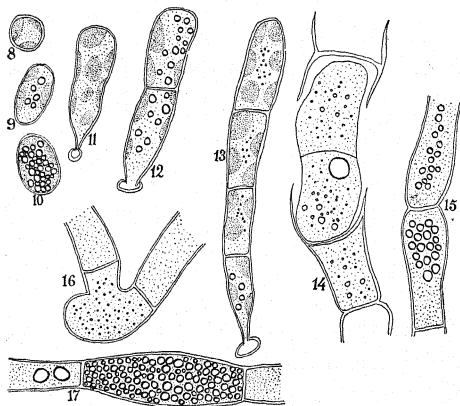


Fig. XII. *Tribonema bombycinum* Derb. et Sol.

8-13. Akinetes forming young plants. In 11-13 a disc of attachment is seen.

14. Filament showing the formation of two aplanospores in a single cell.

15. Filament swelling to form akinetes.

16. "Knee-shaped" form which will probably give rise to an akinete.

All $\times 1200$.

The akinetes fall apart and germinate without resting (Fig. XII, 8-13). Irregularities in the form of the filament often occur in cultures and such swellings may probably become akinetes (Fig. XIII, 19, 20).

Zoospores are formed, two or rarely three or four in each cell (Fig. XIII, 21). Each is broadly pear-shaped and is provided with two very unequal cilia, and a number of fine granules are present (Fig. XIII, 22-26). Movement is very active for a time. Ultimately

the zoospore comes to rest rather gradually, and becomes a filament by cell-division.

There was no evidence of conjugation of gametes as described by Scherffel (37) who failed to see the two cilia. He also records a distinct eye-spot. Hence it is probable that there is some confusion. As Scherffel's observations have never been confirmed it is impossible to regard the reproduction of *Tribonema* by conjugation of gametes as an established fact.

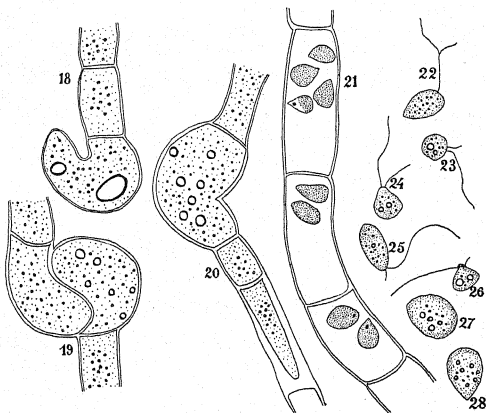


Fig. XIII. *Tribonema bombycinum* Derb. et Sol.

18-20. Swollen filaments which will probably result in the formation of akinetes.

21. Filament containing zoospores.

22. Zoospore with branched cilium (rare).

23-26. Ordinary zoospores with two unequal cilia.

27-28. Zoospores which have come to rest after having lost their cilia.

18-20 $\times 1200$. 21-28 $\times 1650$.

GENERAL REMARKS ON THE HETEROKONTAE

In view of the fact that only five genera have been studied by me in detail, I do not attempt here a classification of the whole group. There have been a number of efforts to do so, but before we can hope for a thoroughly satisfactory arrangement of the series more detailed

knowledge of each genus is required. However, in spite of well-defined generic differences, the above five genera have pronounced structural and physiological features in common, which warrant their inclusion in the same series. The remarks that follow apply largely to these genera, and as far as our present knowledge goes, many of them will be found to apply to other members of the group. It is highly probable that as the knowledge of the other genera is extended these statements will be found to have a still wider application.

Perhaps one of the most prominent features common to all these genera is the presence of numerous discoidal yellow-green chromatophores in the cell. There is no trace of the variety of form or complexity of the chloroplast such as is found in the Isokontae. The chlorophyll is in each case associated with xanthophyll, which is shown by warming the alga on a slide with strong hydrochloric acid. A blue-green colour is invariably obtained. This result is very different in the case of the Chlorophyceae. If the same test is performed on *Protococcus* or *Microspora* for example, the chloroplasts appear green or yellow-green, never blue-green as in the Heterokontae. There is an entire absence of starch, either as pyrenoid, or diffused in the general cytoplasm, the assimilation product being some form of oil which usually appears as fine granules, large or small drops, or whitish semi-opaque bodies as in *Tribonema*¹. The formation of the oily material may possibly be associated with the occurrence of a considerable proportion of xanthophyll in the chloroplast². Not only is this feature strikingly uniform in all the genera, but the assimilation product responds to the same micro-chemical tests even in examples as far apart as *Chlorobotrys* and *Tribonema*. This is true both for the minute granules and also for the larger globules of oily material.

The uniformity of structure of the motile spores is another noticeable feature. Though the zoospores may differ in size, and to some extent in shape, the presence or absence of a pigment spot, and other details, they are invariably characterised by the presence of two

¹ Blackman states that a small amount of hexose may be extracted by water and it is probably the earliest product of assimilation, which is afterwards reduced to fat.

² It is interesting in this connection to note that *Vaucheria* is the only alga among the Chlorophyceae which agrees with these characters, both with regard to the presence of xanthophyll and the formation of oil. It also responds similarly when heated with strong hydrochloric acid. In spite of these resemblances, however, *Vaucheria* cannot be regarded as belonging to the Heterokontae. The peculiar character of the zoospore, and the highly differentiated sexuality sharply separate it from this group.

unequal cilia. This applies both to micro- and macro-zoospores when both are present. The difference in length may be very pronounced, as in *Tribonema bombycinum*, or it may be slight, as in *Botrydiopsis minor*, but the cilia are never of equal length. This is in striking contrast with the zoospores of the Isokontae, which have two or four cilia, always of equal length. This feature is so constant that it has undoubted systematic value (see below, p. 331).

Again, one can scarcely fail to observe the comparative simplicity of the reproductive process. Vegetative division and the formation of asexual motionless spores are common, and all the genera are characterised by the formation of zoospores. As already stated, I have been unable to discern any indication of fusion of gametes, even when, as in *Heterococcus*, micro- and macro-zoospores are formed. In this instance, though hundreds of zoospores were observed, they exhibited no tendency whatsoever to unite. Even in *Tribonema*, the highest known member of the group, no fusion was ever observed. The only original account of a sexual process in *Tribonema* was given by Scherffel (37) who claimed to have witnessed the fusion of gametes, but so far as I have been able to ascertain these observations have never been confirmed. Similarly with Borzi's account of the union of gametes in *Characiopsis* (11) and *Botrydiopsis* (7). When one compares the sexual process in many of the Chlorophyceae, e.g. *Volvox*, *Spirogyra*, etc., the difference appears very pronounced, and should a more extended study of the group confirm the rarity or absence of gametogenesis, then it would appear that the Heterokontae are on a line of evolution quite different from that of the Chlorophyceae.

A very characteristic feature is the lack of regularity that is shown to such a striking degree in the Chlorophyceae. This appears not only in the inequality of the cilia of the zoospores as already stated, but also in their mode of formation. In all the genera studied, with the exception of *Tribonema*, in which probably two to four zoospores are characteristically formed in the zoosporangium, the zoospores are numerous and are formed quite irregularly. There is no indication of the regularity of cell-division which is so common in the Chlorophyceae where two, four, eight, sixteen etc., zoospores are formed as a result of a regular process of cell-division. It seems probable that in *Chlorobotrys*, *Characiopsis*, *Botrydiopsis* and *Heterococcus* the number of zoospores has some reference to the number of chloroplasts originally in the cell, but I cannot state this as a fact.

In the same way, where autospores are formed, as in *Botrydiopsis* and *Heterococcus*, there is not the slightest suggestion of regularity

in their formation. Tetrads are seldom seen. It is possible that the absence or rarity of tetrads is an indication of the lack of sexuality. Although this point has not been definitely proved, it seems to be very likely. Similarly, in cases of vegetative reproduction it is not very common to find a mother cell giving rise to a tetrad of daughter cells. Occasional examples are found in *Botrydiopsis* and *Chlorobotrys*.

This characteristic may or may not be of general occurrence throughout the group. Luther⁽⁵³⁾ states that in the cell-division of *Chlorosaccus* the cells normally form four daughter cells. It would be interesting to see how far this feature is common to the other members of the Heterokontae.

The lack of regularity is also shown where colonies occur. If, for example, the colonies of *Chlorobotrys stellata* are compared with those of many of the Chlorophyceae, e.g. *Pediastrum*, *Scenedesmus*, etc., the difference is at once apparent. The colony in *Ch. stellata* is merely an aggregation of cells held together by pectic material without the slightest regularity of formation¹—a false coenobium.

Among these five genera we have examples of plants with a marked tendency to variability of form, while others exhibit a higher degree of stability. E.g. it has been shown that *Characiopsis ovalis* is extremely variable in shape, size, presence or absence of a stalk and beak, though it always preserves its unicellular condition. *Heterococcus viridis* may be unicellular, or multicellular, when the plant may assume various forms, e.g. plates, filaments (branched or unbranched), etc. *Chlorobotrys stellata*, while always remaining unicellular, and usually more or less spherical in form, may vary in the presence or absence of the stellate wall, which considerably alters the appearance of the cell. These studies have shown the importance of examination of the algae under differing conditions of environment. A mere examination of an alga taken directly from nature, where the environmental conditions may be uniform, is insufficient in view of such variation, and such a proceeding often leads to multiplication of species and consequent confusion. This is especially the case in *Characiopsis*. The study of an alga should also be continued for a considerable period so that all stages in the life-history may be observed. The method of pure culture is admirably adapted for such a study, and is an invaluable complement to the study of the alga taken directly from natural conditions.

Botrydiopsis and *Tribonema* are, unlike the other three genera, far more stable in form. In *Tribonema* the filamentous condition is

¹ Cf. *Ch. regularis* Bohlin (1).

unvarying, the only variations appearing in the knee-shaped or swollen forms already referred to, which occur under certain conditions of environment. Such variations, however, are of local occurrence and do not greatly alter the appearance of the filaments.

With regard to the membrane of the genera studied, two characters stand out prominently, viz. (a) the membrane usually contains a comparatively large proportion of pectic substances, and a small amount of cellulose. *Characiopsis ovalis* is a possible exception. In this case there appears usually a larger proportion of cellulose. Pascher (57) in his recent work (1925) states that the presence of cellulose in the cell-walls of the Heterokontae has not been definitely determined. It is clear from my studies, however, that a certain amount of cellulose is often though not invariably present, even in the walls of mature cells. In the Chlorophyceae, though pectic compounds are often present, e.g. *Spirogyra*, there is usually a relatively larger proportion of cellulose. (b) There is a tendency to a more complex structure of the membrane, e.g. the layering of the wall of *Tribonema*, and the formation of H-shaped pieces with peculiar cross-walls; the stellate form of the walls of *Chlorobotrys stellata* under certain conditions. In *Characiopsis*, *Botrydiopsis* and *Heterococcus* the membrane is of a simpler character. Whether this complexity is associated with the presence of large quantities of pectic substances I have not been able to determine with certainty, though it seems very likely in the case of *Chlorobotrys stellata*. It is interesting to recall that a noticeable complexity of structure is shown also in the membranes of *Ophiocytium*, *Sciadium* and other members of the group.

In certain genera of the Heterokontae the cell-wall is divided into two parts. This may apply to the vegetative cells, or the asexual spores, or to both in some cases. This is an interesting feature and is fairly common, and when our knowledge of the group has extended it is possible that the character may be found to be of far more common occurrence. For example, in *Chlorobotrys regularis*, according to Bohlin (1), the walls of the "cysts" are said to fit together like the two parts of a box. It is possible, however, that Bohlin had here two different algae under observation, the "cysts" being a unicellular species more nearly akin to *Pseudotetraëdron*, *Centrtractus* and others. No such "cysts" have been observed for other species of *Chlorobotrys*. Similarly, Lagerheim (33) has recorded in the aplanospores of *Tribonema* a membrane consisting of two unequal parts, the smaller one becoming detached like a lid when the spore germinates.

Pascher (57) figures double membranes in the spores of *Ophiocytium*, *Chlorothecium*, *Pseudotetraëdron* and *Meringosphaera*, somewhat similar to those of the "cysts" of *Chlorobotrys regularis*.

In the ordinary vegetative cells a double character is visible in *Tribonema*, where each cell is bounded by adjacent parts of two H-shaped pieces; the cells of *Ophiocytium* also show this double character, also those of *Centritractus* and *Pseudotetraëdron*. Pascher mentions in addition a species of *Characiopsis* (*Ch. crassipex*) in which the upper portion of the cell is converted into a small lid which becomes separated from the cell. It is probable, however, that this alga is not *Characiopsis* but is nearer the genus *Ophiocytium*. Nothing of the kind was observed in *Ch. ovalis*.

The siliceous nature of the membrane which is characteristic of *Chlorobotrys* does not appear in the remaining genera studied. However, according to Pascher, silica is found in the cell-walls of a number of Heterokontae.

It is clear on comparing the series with the Chlorophyceae that the differences are considerable, and may be enumerated thus:

The nature of the membrane.

The chromatophores—their form and chemical nature.

The product of assimilation.

The complete absence of starch.

The lack of regularity, e.g. (1) in vegetative division, (2) in the formation of motionless and motile spores.

The structure of the zoospores with two unequal cilia.

The rarity or absence of sexual reproduction.

These characters are so uniform in the genera studied (and, as far as is known, in many other members of the group) that it would be absurd to deny that we are here dealing with a group of plants which are quite distinct in structure and possibly also in origin, from the Chlorophyceae. Indeed they seem to have little affinity with this group¹.

The group Heterokontae was first organised in 1899 by Luther (53), who was struck by the similarity of the flagellar apparatus in algae which were apparently as far apart as *Conferva* (*Tribonema*) and *Botrydiopsis*. It was he who suggested the name Heterokontae² and he regarded the group as equivalent to the Chlorophyceae.

¹ It is probable that various genera which are at present included in the Chlorophyceae will, on further investigation, be found to have a more natural place in the Heterokontean series. Such has already happened in the case of *Characiopsis*, which was for a long time confused with *Characium*.

² Pascher (1925) (57) makes the mistake of attributing the name Heterokontae to Bohlin.

In his paper on *Chlorosaccus* (1899) (53) he gives a diagnosis of the characters of the class, and includes a classification of the whole group. Before this date, however, many investigators had shown the resemblance between various genera. E.g. A. Braun in 1855 (26) drew attention to the strong likeness between *Tribonema*, *Ophiocytium* and *Sciadium*. In 1889 Borzi observed the same thing and united them to the Confervales. Bohlin in 1897 (23) discovered that the resemblance between *Conferva* and *Microspora* was only a superficial one and that these two belonged to widely different classes. He also showed the similarity in the structure of the membranes of *Conferva* and *Ophiocytium*. Later, other genera were added to the series, e.g. *Stipitococcus*, *Peroniella*, *Chlorobotrys*, etc. Pascher in 1912 contributed *Pseudotetraëdron* (58), and in his paper on this new genus he included a classification of the whole group which is made to include twenty-one genera (several of which are acknowledged as doubtful). Before then many attempts had been made to classify the genera, e.g. those of Luther (1899), already alluded to (53), Blackman (1900) (41), Blackman and Tansley (1902) (42). In 1913 Cavers, in his work on *Recent work on Flagellata and Primitive Algae* (45), adopted the arrangement of Pascher in his classification of the group. It cannot be said that up to the present we have a satisfactory grouping of the genera. Different investigators differ greatly among themselves as to the details of classification. Blackman and Tansley (1902) even include the Vaucheriales in the group. As our knowledge of the detailed structure of each genus extends, a satisfactory system of classification will no doubt be devised. The class Heterokontae is now recognised by most authorities as constituting a naturally developed series. Wille (64), however, does not accept the class as separate from the Chlorophyceae and in his account of the Algae in *Die Natürlichen Pflanzenfamilien* the various genera are scattered among the Chlorophyceae with what appears to be a most extraordinary disregard of natural affinities. E.g. *Bumilleria* and *Tribonema* are united with the Ulotrichaceae, while *Mischococcus* and *Botrydium* find a place among the Protococcales. Similarly, Migula (54) in his work on Green Algae refuses to recognise the independence of the series. These two, however, are the only outstanding exceptions.

It may be asked how far it is justifiable to found the group on the characters of the cilia—how far such a feature is important as a basis of classification. From what has been stated above, however, it is seen that this very uniform characteristic is associated in these

five genera with others, equally striking, such as the form of the chloroplasts, their chemical nature, and the assimilation product. As investigation proceeds it is becoming more and more clear that the group is far from being the artificial assemblage it was once thought to be. Hence it cannot be alleged that too high a value is accorded to a character which seems to be an insignificant one. In this connection I cannot do better than quote the words of Chodat¹: "Admettons avec Wille que le nombre des cils à lui seul ne suffise pas comme caractère principal de classification. Mais si, à ce caractère du nombre et position des cils, viennent s'adjoindre d'autres caractères qui le complètent, ce caractère prend une importance capitale." Further, "utiliser pour la classification un caractère choisi n'est pas faire de la classification artificielle, si ce caractère est lié, s'il est en rapport constant avec d'autres caractères." It is perhaps significant that there appear to be no transitional forms between zoospores which have the different types of cilia (four, all equal; two, equal; two, unequal; a crown of cilia as in *Oedogoniaceae*; cilia scattered over the whole surface of the zoospore as in *Vaucheria*), unless a variation in the relative length of the short cilium in *Heterokontae* can be so regarded (cf. *Botrydiopsis* and *Tribonema*).

There is no evidence to suggest that the short cilium is due to a degeneration of an originally long one. A curious variation was once observed in a zoospore of *Tribonema bombycinum*, in which the shorter cilium appeared to be a branch of the longer one (Fig. XIII, 22). Luther⁽⁵³⁾ considers that in *Chlorosaccus* it is the longer cilium which effects the greater part of the movement of the zoospore, but I have no evidence to support this in the case of the genera I have studied. It seems unlikely that this would be the case where there is little difference in the lengths of the cilia as in *Botrydiopsis*. On the other hand, it may readily be imagined where there is considerable disparity in the length of the cilia, as in *Tribonema bombycinum*. This is also the case in *Chlorosaccus*. Luther in his figures shows that the short cilium is one-third to one-quarter the length of the longer one.

It has been suggested by Chodat that the *Heterokontae* may probably be found to have a flagellate ancestry. Pascher⁽⁵⁶⁾ considers that *Chlorophyceae* may begin with *Polyblepharidae* and *Volvocaceae*: so in the *Heterokontae* we may discern a flagellate beginning, leading to the simpler and thence to the more complex forms. Many of the *Heterokontae* may be regarded as parallel to similar forms in the *Chlorophyceae*, the general direction of de-

¹ (46), p. 239.

velopment being broadly similar in both groups. Thus, for example, *Chlorobotrys*, *Botrydiopsis* and *Characiopsis* represent approximately the same level as many of the Protococcales, while *Tribonema* and *Heterococcus* correspond more nearly to the Ulotrichales.

Pascher agrees with Chodat that the most primitive of the Heterokontae have undoubted flagellate characters. These he includes in the term Heterochloridales, though other authors, e.g. Blackman and Tansley (1902) (42), prefer to consider them as Flagellates, not Algae.

The class includes two genera, *Chloramoeba* and *Stipitococcus*. *Chloramoeba* was described by Lagerheim in 1897 and may be regarded as derived from flagellates of the *Chloromonadina* type, which have been shown to possess numerous disc-like, yellow-green chloroplasts, e.g. *Vacuolaria*. Blackman, however, regards *Chloramoeba* as a typical flagellate. The peculiar interest of this form lies in its undoubted rapport with the Heterokontae, e.g. it is provided with two cilia, a long one, and a very short one, which is often curled against the body. The chloroplasts are discoidal, two to six in number, yellow-green, and give a marked blue-green colour when treated with strong hydrochloric acid. It is strikingly similar to the typical Heterokontean zoospore. In addition to its active movements it exhibits also amoeboid movements. It is interesting here to notice that amoeboid movements of the zoospores of certain Heterokontae, e.g. *Tribonema*, *Botrydiopsis*, have been recorded by several observers (e.g. Lagerheim (33) and Borzi (7)). Whether *Vacuolaria* is regarded as a true flagellate or as a primitive alga of the Heterokontean series makes no difference to the view that it occupies an interesting position as a transition between true flagellates and the Heterokontae. It appears to be almost equally balanced between the two groups.

Chlorosaccus, which was described by Luther (34) in 1898, is of importance in forming a link with *Chloramoeba* and the higher forms of Heterokontae. While retaining certain flagellate characters, e.g. the cell-division takes place parallel to the long axis, it is non-motile in the vegetative state, and becomes motile only in the zoospore condition.

The presence of silica in the membranes of certain Heterokontae is probably a further link with the Flagellata. Cysts containing silica are characteristic of several flagellates whose existence has been shown by Klebs. It is remarkable also that the bivalve structure of the wall is also found in certain of the Flagellata. The

unicellular organism described by Bohlin as the "cyst" of *Chlorobotrys regularis* is interesting in this connection. Not only is the cell-wall highly siliceous but it has a distinctly bivalve character. Ultimately, the two halves are said to separate, and two spores are liberated. These are distinctly flagellate characters, and this organism affords an interesting link with higher bivalve forms of Heterokontae such as *Centritractus*, *Pseudotetraëdron*, etc.

It may be urged that as the presence of silica in the cell-wall and also the bivalve character are found in groups which are far removed from one another, the connection of the Heterokontae with the Flagellata, based on these grounds, should not be insisted upon. It is true that these resemblances alone would be insufficient on which to base a theory of the origin of the group. Such considerations, however, must be taken along with the study of the zoospores as suggested above, and they undoubtedly help to strengthen the impressions so gained. As long as the zoospores of various Heterokontae, e.g. *Tribonema*, *Botrydiopsis*, etc., were regarded as possessing one cilium only, this formed a serious objection to the theory that the class was derived from flagellate ancestors having two unequal cilia. Since then, however, Luther's discovery of the shorter cilium in *Tribonema* and *Botrydiopsis*, removed this objection as far as it applied to these two genera. As I have shown in the preceding studies this character is found also in *Chlorobotrys*, *Characiopsis* and *Heterococcus*. No doubt as observations are extended over the whole group, this character will be found to be a constant feature, and the origin of the Heterokontae from flagellate ancestors will then appear to be well established.

Chodat, who firmly believes that the Heterokontae are entirely distinct from the Chlorophyceae, suggests^{(46), (47)} that they find a place in the same series as the Phaeophyceae owing to the analogy between the Ectocarpaceae, especially in the structure of the zoospores, and the greater part of the brown or yellow flagellates. The presence of a secondary pigment is another feature common to the members of these groups. Not far from these, and possibly of similar origin, Chodat places the Diatomaceae with their bivalve siliceous shells, and the Peridiniae, whose motile vegetative condition connects them still more closely with the Flagellata. Both these groups have characters which suggest a relation through the Flagellates, with the Heterokontae and the Phaeophyceae.

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MYCORRHIZA

By M. C. RAYNER

CHAPTER VIII

Ectotrophic mycorrhiza: Müller; Möller; McDougall—Peyronel: the fungi responsible for forming mycorrhiza in trees—Melin: the mycorrhiza of Pine, Spruce, Larch, Aspen and Birch; the ecological and physiological significance of tree mycorrhiza; application of experimental results to field conditions and forestry—Falck's work on the decomposition of humus.

AFTER the publication of Stahl's paper on the subject in 1900, interest in mycorrhiza was transferred temporarily to plants showing the endotrophic type of infection. The first fifteen years of the new century were comparatively rich in researches dealing with the cytology of intracellular infection and saw also the application of new and fruitful methods of experiment to the study of such specialised groups as the Orchids and Heaths. The possible importance of the ectotrophic type of mycorrhiza, characteristic of so many forest trees, as a factor affecting growth continued, however, to attract attention. A classical contribution to the subject was made by Müller (1903) who published an account of his observations on Spruce and Mountain Pine in Jutland.

Spruce is an important forest tree in the heath region of Jutland, and widespread failure in its culture had attracted the attention of foresters. After a satisfactory start, the trees suffered a severe check, as evidenced by diminished annual growth, decreased size of the needles and dying back of the tops. Whole plantations suffered in this way, either dying off completely or making a slow recovery—in either case causing considerable loss of revenue. Clearing the land of heather before planting and better cultivation caused no improvement, but good results were obtained by planting the Mountain Pine, *Pinus montana*, as a nurse tree in young plantations of Spruce. Müller sought an explanation of this fact and, after examining the factors likely to be operative, fixed upon the mycorrhiza of the Pine as the chief agent responsible for the improvement. He had previously described and figured two forms of mycorrhiza in the Mountain Pine (1902): (a) a racemose form with

ectotrophic mycorrhiza, and (b) a dichotomous form with endotrophic mycorrhiza: in Spruce, on the other hand, he observed only a form with racemose branching and ectotrophic structure. Müller argued that just as Clover and other leguminous species indirectly provide nitrogenous food for other plants by means of their root tubercles, so the Mountain Pine with its endotrophic mycorrhiza, furnished to the Spruce nutrient material inaccessible to the latter when growing alone. Noting that heather and other heath plants also possessed endotrophic mycorrhiza, he inferred that heath conditions were better suited to the Mountain Pine than to Spruce.

If proved, this interesting hypothesis would have an important bearing upon practical forestry. Unfortunately, Müller failed to obtain the requisite experimental proof, while Möller (1906), to whom he supplied seeds and seedlings of *P. montana*, reported that he could find no evidence for fixation of atmospheric nitrogen by the mycorrhizal fungus.

The last-named worker had published two papers (1903 a, b) recording his observations on the growth of Pine (*P. sylvestris*) and Oak, in poor sandy soils and in those containing abundant humus respectively. His attention was attracted by the coralloid mycorrhiza on the roots of Pine (*P. sylvestris*), and he made various attempts to determine its relation to the nutrition of the tree. Frank (1892, 1894) had previously expressed the opinion that this conifer did not come to maturity on good Pine soils if the formation of mycorrhiza was hindered through absence of the appropriate fungus, whereas it grew vigorously if the roots were infected normally. Möller found experimentally that a direct relation existed between luxuriant development of the trees and abundance of mycorrhiza, but his experiments provided no evidence whatever that the root fungi of either Pine or Oak were able to utilise atmospheric nitrogen. Nor were his efforts to establish the identity of the mycorrhizal fungi more successful, the technique adopted rendering it little probable that the species of *Mucor* isolated from sterilised fragments of root were actually the forms concerned in the formation of mycorrhiza, as indeed was clearly recognised by the investigator himself. He quoted Ramann's experiments (Ramann 1888) which had given similar results, and recorded his own conclusions in the form of an extract from a communication made to him by Sarauw:—"Dass die Pilz unseren Waldbaumwurzeln und den Bäumen Vorteil bringen sollten, ist bisher meines Erachtens weder durch Beobachtungen in der Natur, noch durch Versuche nachgewiesen worden."

In a paper on the mycorrhiza of forest trees, McDougall (1914) described the use of an improved technique for the identification of the fungi present in the mycorrhizas of various trees. It will be recalled that Noack (1889) had put forward a claim for their identity with certain Gasteromycetes and Hymenomycetes commonly found in the neighbourhood of the trees. Noack based his conclusions on the microscopic characters of the mycelia and the frequent association of particular fruit bodies with certain trees. Using the "glass plate" method, McDougall kept individual roots under observation throughout the growing season and, in the paper cited, offered substantial evidence for the identification of the root fungi in six forms of ectotrophic mycorrhiza, viz. *Russula* sp. on *Tilia americana*, *Boletus scaber* var. *fuscus* and *Cortinarius* sp. on a variety of *Betula alba*, and *Scleroderma vulgare* on *Quercus alba*, thus adding four new species to the list of fungi for which claims had been put forward previously. He observed the course of infection and followed the development of the characteristic ectotrophic structure described by Frank, noting the progressive intensity of external infection and its direct correlation with the inhibition of root growth and stimulation of branching which resulted in the development of coral-like clusters of mycorrhiza. McDougall described and figured a number of typical cases and recorded two novel structures, one, a curious type of infected root in Maple with bead-like swellings and a strict localisation of mycelium to the swollen regions, the other a development of what was described as *heterotrophic* mycorrhiza in *Tilia americana*—ectotrophic in general structure, but showing intracellular infection of the cortical cells. He believed that the mycorrhizal relation in trees was a casual one, depending upon the proximity of a suitable fungus or on some chance condition in one or other of the constituents. In respect of the physiology of the relation, he argued that among the endophytic mycorrhizas found in Maples some were associations beneficial to the trees, whereas in others the fungi were simply internal parasites of the roots. The true ectotrophic mycorrhizas of the majority of trees he regarded, without exception, as cases of parasitic attack on the roots, adding in a later paper (1922):—"It is probable that as a rule no great harm to the higher plant results from this parasitism of its roots by mycorrhizal fungi."

The confusion existing at this time with regard to the identity of the root fungi of trees may be gleaned from the following brief summary: members of each of the great groups, Basidiomycetes, Ascomycetes and Phycomycetes, had been cited by different ob-

servers as responsible for mycorrhiza formation. Among Basidiomycetes, Woronin (1885), Noack (1887) and Frank (1892) had each identified a number of Hymenomycetes as mycorrhizal fungi; Kaufmann (1906), Pennington (1908), Mimura (1915), McDougall (1914, 1922), Romell (1921), and Peyronel (1920, 1922) added progressively to the list. Boyer (1915-16), tracing the delicate mycelium of species of *Boletus* and *Amanita* through the soil, observed that it could frequently be connected with the mycorrhiza of the tree roots. He believed the same to be true of truffles and other humus-dwelling fungi. Of Gasteromycetes, Noack (1889) had cited *Geaster* sp.; McDougall and Peyronel *Scleroderma vulgare*. Among Ascomycetes the association of truffle fungi with the roots of certain trees had long attracted attention; Mattiolo (1887) and Frank (1892) both believed that species of *Tuber* were mycorrhiza formers; Reess (1880, 1885 a) and others had held a similar view for *Elaphomyces granulatus*; Pirotta and Albin (1900) made a new claim for *Terfezia leonis*.

Peklo (1909) contributed an account of his researches on the ectotrophic mycorrhizas of *Carpinus* and *Fagus*, including attempts to extract the root fungi and establish their identity. From roots of both trees he isolated forms of *Penicillium* and *Citromyces* and attempted to prove their identity with the mycorrhizal forms by inoculation of mycorrhiza-free cultures of young Beeches with spore suspensions. The technique adopted by Peklo was somewhat crude, and it is probable that the strains of "Wald-Penicillien" isolated by him were merely members of the profuse epiphytic fungus flora upon the roots used for isolation experiments.

As representatives of the Phycomycetes, Möller (1903) had added the names of several species of *Mucor* extracted from the roots of Pine (*Pinus sylvestris*). In this case, as in the last mentioned, it is unlikely that the forms isolated were other than casual associates of the superficial microflora of the roots. Fuchs (1911) made similar attempts to establish the identity of the root fungi of certain Conifers by the addition of spores of *Fusarium* sp. and *Verticillium* sp. His efforts to reproduce ectotrophic mycorrhiza by these means were unsuccessful. The immediate cutting off of the infected cells observed was interpreted as evidence of parasitic attack on the part of the fungi concerned.

For the inclusion of a majority of the fungi in this rather heterogeneous group, the main evidence consisted of observations on a regular association of particular fruit bodies with the trees concerned; on the continuity of mycelium from the latter with the hyphae of the fungus

mantle on the roots; and on the morphological identity of the hyphae attached to sporophores and roots respectively. Although inconclusive, evidence of this nature was cumulative and suggestive in respect to certain Hymenomycetes, more especially in regard to certain species of *Boletus*; the constancy of the records for an association of members of the Tuberaceae with various Cupuliferous trees may also be noted. Certain observers, e.g. Peklo and Möller, had undertaken investigations by means of pure culture methods. In no single case, it may be safely said, had experimental evidence of a satisfactory kind been obtained that the fungus named was actually concerned in the formation of ectotrophic mycorrhiza.

The old problem of the relation of Truffles to forest trees is recalled by certain recent researches on the behaviour of certain species of *Tuber* in pure culture. Matruchot had grown *Tuber melanosporum* and *T. uncinatum* on artificial media and reached the conclusion that these species depended upon an association with Oak for the production of fertile ascocarps. Constantin (1924), on the other hand, has since been successful in obtaining cultures of *T. brumale* which produced both conidia and ascospores on artificial media quite independently of any relation with roots of Oak. The association of fungi of the Truffle series with the mycorrhiza of other trees remains an open question; synthetic cultures recently carried out by Melin with *Elaphomyces* sp. have yielded uncertain results.

The observations of the Italian mycologist Peyronel (1922 a, b) on the association of various Basidiomycetes with certain trees have led him to publish the following list as representing almost certain cases of mycorrhizal association.

Fagus sylvatica

Cortinarius proteus
C. bivelus
Boletus cyanescens
B. chrysenteron
Hypochmus cyanescens (nov. sp.)
Scleroderma vulgare
Amanita rubens
Lactarius subdulcis
L. blennius
Russula emetica
R. nigricans
Cantharellus infundibuliformis
Hydnum repandum
Lactarius coryli (nov. sp.)
L. subdulcis

Corylus avellana

<i>Corylus avellana</i>	<i>Boletus chrysenteron</i> <i>Strobilomyces strobilaceus</i> <i>Hypochmus cyanescens</i> (nov. sp.) <i>Amanita rubens</i> <i>Rhodopaxillus nudus</i> <i>Cortinarius proteus</i> <i>C. multiformis</i> <i>C. violaceus</i> <i>Hydnum repandum</i>
<i>Betula alba</i>	<i>Amanita muscaria</i> <i>Amanitopsis vaginata</i> <i>Lactarius necator</i> <i>L. torminosus</i> <i>Boletus scaber</i> <i>Scleroderma vulgare</i> <i>Russula rhodoxantha</i> (nov. sp.) <i>Tricholoma flavobrunneum</i>
<i>Larix decidua</i>	<i>Amanita muscaria</i> <i>Russula laricina</i> (nov. sp.) <i>Hygrophorus bresadolae</i> <i>H. lucorum</i> <i>Scleroderma vulgare</i> <i>Lactarius rufus</i> <i>Gomphidius gracilis</i> <i>Paxillus lateralis</i>
<i>Populus tremula</i>	<i>Cortinarius collinitus</i>
<i>Quercus robur</i>	<i>Amanita citrina</i> <i>Lactarius subdulcis</i> <i>Russula cyanoxantha</i>
<i>Castanea vesca</i>	<i>Amanita rubens</i> <i>Russula lepida</i> <i>R. rubra</i> <i>Scleroderma vulgare</i>

Peyronel has pointed out that the general form and dimensions of the various mycorrhizas are determined by the host tree, while the structure, thickness, colour, etc., of the mycelial mantles depend upon the individual fungi present. It is stated, for example, that Larch mycorrhiza formed by *Scleroderma vulgare* is macroscopically indistinguishable from that formed by *Boletus elegans* in the same tree. In certain cases macroscopic characters are said to afford a ready means of identification; thus, the mycorrhiza of beech formed by *Hypochmus cyanescens* is immediately recognisable owing to its fine blue colour. Peyronel has bestowed the new name "mycoclana" upon the fungus mantle, and predicts that it will be possible eventually to make an analytical key which will permit the immediate

identification of the fungi present in any mycorrhiza by observing the characters of the mycelium.

Peyronel has been led to make the new and somewhat remarkable claim that certain Hymenomycetes show constant morphological differences when associated with different trees. For example, the sporophyte of *Boletus scaber* associated with Birch is reported as different from that of the same fungus growing with Hazel, Oak, and Chestnut. Some of the opinions expressed by this author will be received with great caution by mycologists, especially by students of the Agaricineae.

From observations on the west coast of Sweden, Romell (1921) was struck by the constant presence of "fairy rings" of the "Butter Fungus," *Boletus luteus* around *Pinus montana*, independently of soil differences. A similar association had been observed elsewhere, not only with the Mountain Pine, but also with *P. austriaca* and *P. sylvestris*. Earlier, Quélet (1888) had reported *Boletus boudieri* in association with *Pinus halepensis* and *Pinus pinaster*, and *B. elegans* with *Larix*. Attempts to trace a direct connection of the mycelium of *Boletus luteus* with the roots of *P. montana* did not yield conclusive evidence, but Romell's conviction that this existed, as also his view in respect to the significance of the association between *Boletus elegans* and Larch, have been fully justified by subsequent researches.

The close association of a "fairy ring" of Hymenomycete sporophores with a given species of tree occasionally observed in nature is illustrated in the photograph reproduced in Fig. 52, although in this case the identity of the species of *Clitocybe* to which the fructification belongs with any of the fungi present in the mycorrhiza of the tree was not investigated.

In a brief account of mycorrhiza in Sitka Spruce (*Picea sitchensis*) and other trees, Laing (1923) has supplied an analytical table of the various types of mycorrhiza observed by him in conifers and noted that both the ectotrophic and endotrophic types of structure are represented as well as an intermediate or "semi-ectotrophic" type. *Thuja gigantea*, *Sciadopitys verticillata*, and *Pinus sylvestris* are cited as forms showing intracellular infection. In discussing the unsatisfactory character of the evidence relating the mycorrhiza of trees to the intake of food material, Laing describes certain observations of his own indicating the presence of relatively large amounts of oxidising enzymes—oxidases and peroxidases—in the mycorrhiza of trees, and discusses the possible advantage of this increased

oxidising mechanism to the trees in habitats of deficient aeration and available plant food. In an account of field observations in south-east England, Paulson (1924) has recently drawn attention to the profuse development of tree mycorrhizas in the superficial rooting system that does not penetrate the soil but ramifies throughout the accumulations of fallen leaves near the trees (Fig. 51). This distribution, if general, may possess some significance in relation to Laing's observations on the presence of oxidases.

Of other coniferous mycorrhizas, that of *Abies firma* was described by Tubeuf (1896), Noelle (1910), and Mimura (1917), none of whom put forward any views as to the identity of the fungus or fungi responsible. More recently, Koki Masui (1926) has observed sporophores of *Cantharellus floccosus* attached to the mycorrhizas of this conifer, and so added another species to the growing list of Basidiomycetes believed to be responsible for mycorrhiza formation. The Japanese observer distinguishes four distinct types of mycorrhiza in this Fir, one being an ectotrophic form with curious basidia-like projections from the mantle, an intercellular "Hartig net" of the usual kind, and more or less profuse intracellular infection. The mycorrhizas associated with *Cantharellus* were found only in the superficial layer of soil, and the fungus was observed to occur only in dry situations. Infection reduced the rate of growth of the roots, and was sometimes fatal. The author agrees with McDougall in regarding the relation as essentially one of parasitism on the part of the root fungus. In a later paper (Koki Masui 1926 b), he has described and figured the renewed growth observed in these and similar mycorrhizas after the formation of the mantle. The mycelial sheath splits in several directions and a new fungal investment develops from the margins of the fissures.

Of recent attempts to identify the mycorrhizal fungi of trees by isolation methods may be mentioned that of Chan (1923), who isolated a non-sporing mycelium from roots of Beech growing in Munich and in Tyrol and named it *Mycelium radialis fagi*. It is reported that under certain conditions of nutrition—e.g. the presence of one per cent. peptone in the culture medium—the hyphae of this form become shortly jointed like those of the Orchid fungi. On various grounds, the mycelium was believed to be that of the mycorrhizal fungus of Beech, but no experimental proof of its identity has yet been obtained.

In respect to the significance of mycorrhiza in trees, it may be recalled that every imaginable view has found supporters.

Gibelli (1883), Sarauw (1893), Möller (1903), Pastana (1907), Ducomet (1909), Fuchs (1911), McDougall (1922) and Koki Masui (1926) have regarded all or certain of the root fungi as more or less mischievous parasites. Henschel (1887) reached the conclusion that all Spruces developing unhealthy symptoms possessed mycorrhiza, that the degree of damage to the tree was proportional to the amount of infection, and that the strongest saplings were fungus-free. He concluded that the effect was without exception damaging to the young plants:—"Dass die Einfluss dieses Symbioten auf der Entwicklung der jungen Fichtenpflanze als ein absolut schädlicher angesehen werden müsste." On the other hand, Frank (1892), Stahl (1900), Müller (1903), Tubeuf (1903), Elenkin (1906-1907) all believed the association to be one of beneficial symbiosis.

Efforts have been made to study the physiological relations by chemical and microchemical methods. Weyland (1912) made a serious attempt to review the evidence bearing on the nutrition of mycotrophic plants in general, and was the first observer to use microchemical methods for studying the distribution of inorganic nutrients—phosphorus, potassium and calcium—in the root cells. From his own observations on the roots of autotrophic and mycotrophic plants he believed that these methods offered the most promising line of research for gaining new information as to the nutritive relations of members of the latter group with their fungal associates. His conclusions with respect to ectotrophic mycorrhiza agreed with those expressed by Fuchs (1911), a contemporary observer, viz. that the root fungi of trees were parasitic upon their hosts and that no symbiosis of a kind beneficial to the latter existed.

Weevers (1916), examining the distribution of ammonium salts, discovered that these were present in very small amounts or were entirely lacking in mycorrhiza plants. Hence, he argued, assimilation of nitrogen, if facilitated by the root fungi, must be brought about in a different manner from that in leguminous tubercles, in which these salts are present in quantity. More recently, Rexhausen (1920) carried out observations on a number of ectotrophic mycorrhizas by similar methods. Using as material species of *Pinus*, *Quercus sessiflora* and *Monotropa hypopitys*, growing in the open, he reached the conclusion that the root fungi facilitated the absorption of mineral salts, i.e. he found evidence in support of the Stahl theory of nutrition. Rexhausen also expressed the opinion that the relation between fungus and root in ectotrophic mycorrhiza was not a fixed one, but fluctuated with the soil conditions. If these were unfavourable to

the fungus, it might parasitise and greatly injure the tree, if they were too favourable, infection was feeble and mycorrhiza badly developed. On the whole, in the coniferous trees studied, he found evidence that a symbiosis of a beneficial kind existed, especially under soil conditions such that a supply of carbohydrates outside the roots was associated with a deficiency of mineral salts. His observations upon *Monotropa* did not throw any fresh light upon the problem of nutrition in this species.

The recent researches of Elias Melin (1917-1925) mark a notable advance in the knowledge of ectotrophic mycorrhiza. By the publication of this series of papers the author has provided, for the first time, reliable and conclusive evidence of the nature and constitution of the mycorrhizas of certain trees and the identity of the fungi concerned. The work is of such interest and importance that its consideration at some length calls for no apology.

In a brief preliminary note on the mycorrhiza of Scotch Fir and Spruce, Melin offered the following new facts for consideration. (a) The fungi present in ectotrophic mycorrhiza are not strictly intercellular in distribution as was claimed by Frank; the mycelium invades many of the cortical cells and can be put in evidence by suitable staining methods. (b) In addition to the normal ectotrophic type of mycorrhiza in Pine and Spruce, there appears on the roots of individual trees growing in wet bogs and occasionally also in raw humus elsewhere, a fungus association of a different character in which the "Hartig net" and mycelium mantle are lacking and the mycelium is exclusively intracellular. (c) Two fungi concerned in the formation of the mycorrhiza of Pine and Spruce have been isolated and their identity established by means of inoculation cultures; provisionally they were named *Mycelium radialis silvestris* and *Mycelium radialis abietis* (Melin 1922). A full account of these important researches was published subsequently in the form of a monograph and will now be considered in greater detail. (Melin, 1923.)

The more significant results fall naturally under three headings:—

(1) An account of mycorrhiza in Pine and Spruce as it occurs in nature.

(2) The isolation, cultivation and description of the root-fungi; and the reconstitution of the mycorrhizas under pure culture conditions.

(3) The experimental identification of the mycelium of certain root-fungi with Hymenomycete fructifications associated regularly with the trees in nature.

A. THE MYCORRHIZA OF PINE (*Pinus sylvestris*)
AND SPRUCE (*Picea abies*).

In conifers, as in most deciduous trees, it is the actively absorbing roots which become mycorrhizas. The differentiation of the root system into "long" and "short" roots is thereby greatly accentuated, the distinction between the two types being much less when they are protected from fungal infection. In Sweden, mycorrhiza is formed in spring and autumn, the periodicity corresponding with that of root growth showing two maxima, in spring and autumn respectively. Several types have been observed in Pine and Spruce, differing in mode of branching, colour and internal structure.

For the former tree, Melin has described three forms:—"Gabelmykorrhiza," "Knollenmykorrhiza" and simple mycorrhiza ("einfach Mykorrhiza"). The first type, noted and recorded by many earlier workers, is the commonest; it is best developed in woodland soils with an abundant layer of raw humus, but is found also on moorland soils, especially after drainage. The colour varies from golden brown to black while other tints have been observed, the colour differences depending upon the fungi present. The habit of this type shows well the arrest of growth and crowded dichotomous branching so often described, leading to the production of a dense tuft or "witches broom" of characteristic appearance. According to Melin, a majority of root-fungi form this type of mycorrhiza in nature (Fig. 50).

"Knollenmykorrhiza" of Pine is also abundant in Swedish woods on similar types of woodland soil. It presents the appearance of small tubers, variable in size and often so crowded that they have grown together. The tuberous habit is due to the merging of a cluster of dichotomously forked roots into one structure by fusion of the individual fungus mantles. The colour, at first pale, becomes grey to brownish grey with age. The surface of the tubers is rough, owing to the passage outwards from the mantle of strands of mycelium that can be traced into the humus, where they break up into separate hyphae (Fig. 50). Not the least interesting aspect of Melin's work has been the definite association of this type of mycorrhiza with the species of *Boletus* responsible for its formation, viz., *B. luteus*, *B. granulatus*, *B. variegatus* and *B. badius*. He concluded it to be identical with the forms described by Müller (1903) for *Pinus montana* in Jutland and by McDougall (1922) for *P. strobus* in the United States. Both this and the preceding type may occur in close proximity on the same main root (Fig. 50).

The simple mycorrhizas of the Pine are frequently young stages of one or other of the types just described. Sometimes, however, they are believed to represent a special type, in which case they are longer (10 mm. as compared with 4 mm.) and thinner (0.2 mm. as compared with 0.4 mm.). These unbranched mycorrhizas are characteristic of Pine heaths and their formation is believed to depend upon a decreased "virulence" of the root fungi under the conditions existing in heath soils.

Two types of mycorrhiza in Spruce (*Picea abies* L.) have been described; the racemose (razemose) and the simple (einfach) (cf. Müller, p. 339). The former is the characteristic form under favourable soil conditions. The branching is of monopodial type with lateral roots in two rows upon a main axis, fungus infection being similar in the roots of both orders; free branching may produce a cluster of short roots very different in appearance from the typical "Gabelgebüsche" of Pine. As in the latter, the colour is variable, with often a close association of different colours on the same root. The simple type of mycorrhiza resembles that of Pine.

The development and structure of the fungus mantle in these mycorrhizas of Pine and Spruce were found to be very variable, a fact afterwards correlated with soil conditions and the presence of different fungus constituents. For example, in the *Boletus* mycorrhizas, a thicker mantle was formed than with other root fungi, and, in general, the mantle is better developed in the raw humus of Pine woods than on heath soils. The significance of these observations will be discussed hereafter.

The histological characters of the ectotrophic mycorrhizas of conifers have been variously recorded as agreeing with those originally described by Frank (1885) with fungus mantle and intercellular "Hartig net," or showing a truly endotrophic structure as described by Sarauw (1893), or of ectotrophic type with a profuse development of intracellular mycelium as recorded by Peklo (1913) for both Pine and Spruce. These inconsistencies are ascribed by Melin to two causes,—faulty technique on the one hand, and the existence of actual differences correlated with the presence of different root fungi and (or) variation in soil conditions, on the other hand. According to the last named author's observations two kinds of "Gabelmykorrhiza" occur in Pine, one of true ectotrophic type, the other with heavy endotrophic infection of the cortical cells. Noteworthy features in the former are the development of a conspicuous "tannin layer" from the epidermal and sub-epidermal layers of the root, the

cells of which are constantly penetrated by fine hyphae, as well as the presence of a granular layer (Körnerschichte), comprising two or three layers of cortical cells filled with granules of varying size and surrounded by the pseudotissue of the "Hartig net" but otherwise free from fungal infection. In the other form of this mycorrhiza, the "tannin layer" is less conspicuous, and the granular layers are replaced by a region of profuse intracellular infection in which the mycelial contents undergo rapid and complete digestion, the cytological features of the digesting cells resembling those found in Orchids in respect to increased size of the nuclei and the ultimate removal of the stainable products of digestion.

Both types of mycorrhiza, especially when old, are subject to structural modification owing to the attack of a pathogenic fungus, subsequently isolated and named *Mycelium radidis atrovirens*. It is suggested by Melin that the parasitic hyphae of this pathogen were mistaken by Peklo (1913) for those of the true symbiont, and also that the same mycelium was erroneously described as belonging to species of *Cladosporium* by this author.

The "Knollenmycorrhiza" of Pine

The individual roots of the tuberous complexes formed in this type of mycorrhiza are distinguished structurally by the large size of the "cells" of the pseudoparenchyma of the mantle. They are also remarkable for the profuse and typical endotrophic infection of a five- or six-layered cortical region, each cell of which shows active digestion of the intracellular mycelial complex with subsequent disappearance of the stainable products. Without discussing the anatomical evidence in greater detail, it may be stated that Melin regards the structure of all three types of mycorrhiza in Pine as indicating an exchange of food material and hence, pointing to the conclusion that they constitute a working case of mutual symbiosis—"Wurzel und Pilz leben in mutualistischer Symbiose." The microchemical evidence obtained by Rexhausen (1923) is cited in support of this view. A critical consideration of its correctness or otherwise may be postponed to a later chapter in view of the recent publication of the results of an experimental enquiry into the same matter. (Melin 1925.)

The Mycorrhiza of Spruce (Picea abies L.).

Both the structural types of "Gabelmycorrhiza" found in Pine are represented in Spruce, but there is no counterpart in the latter of the "Knollenmycorrhiza" just described. The mycelium of the true symbionts occurs both in the long and short roots of Spruce but was not found in the older main roots. As in Pine, the mycorrhiza of Spruce is subject to the attack of a parasitic fungus which was identified later with that named *Mycelium radialis atrovirens* by the author.

From the foregoing brief account it is apparent that two distinct anatomical types of mycorrhiza are formed in conifers. Melin questioned the propriety of including those showing both intra- and inter-cellular infection with the true ectotrophic forms, and in view of the fact that the former combine the characters of ectotrophic and endotrophic types, he named them *ectendotrophic* (*ektendotrophic*). Mycorrhiza of this composite type was known to occur also in *Larix* (Melin, 1922), and *Betula* (Melin, 1923), and from the literature may be assumed likewise in *Pinus cembra* (Tubeuf, 1903), and *Tilia americana* (McDougall, 1914).

Comparing it with that described by McDougall in *Tilia*, the designation *heterotrophic* used by the latter author was rejected by Melin on the grounds that the word has a very different significance in other connections. It was probably mycorrhiza of this type that Peklo (1913) described for Pine and Spruce in Bohemia, but Melin could find no evidence that a species of *Penicillium* is concerned in its formation, as believed by the former observer.

Mycelium was not observed in the aerial parts of either Pine or Spruce, and attempted isolations from the shoots gave negative results.

Pseudomycorrhiza.

The existence of a "false" mycorrhiza in both Pine and Spruce had been previously recorded (Melin, 1917), the fungus constituent being regarded in general as a one-sided parasite, although the relation fluctuated with soil conditions and might result either in defeat of the fungus by unusually active digestion or in death of the invaded cells. These pseudomycorrhizas are usually simple and unbranched and in Sweden are specially characteristic of moorland soils afforested after draining. Their recognition offers a reasonable explanation for inconsistencies in the records of other observers, e.g. those of Möller

in Brandenburg. It is not improbable that the types of root-infection recorded by Möller from this district were pseudomycorrhizas of similar character.

*The Mycorrhizal Fungi of Pine and Spruce and the
Synthesis of Mycorrhiza.*

Isolation of the fungi responsible for forming the types of mycorrhiza just described, with proof of their identity by the synthesis of mycorrhiza under "pure culture" conditions, constitute an essential part of Melin's researches.

A constant association of the mycorrhizas of Conifers with the fruit bodies of certain Basidiomycetes had been reported by numerous observers, although experimental proof of the continuity of the two systems of mycelia was lacking in every case. The long and heterogeneous list of fungi named in this connection has already been noted and the pressing need, as Melin quickly recognised, was for synthetic cultures on similar lines to those successfully used in the experimental work on *Ericaceae* and the Orchids. Two variations of such methods are available: one, the isolation of the appropriate fungi from roots and the re-synthesis of mycorrhiza under "pure culture" conditions; the other, the formation of mycorrhiza under such conditions by inoculation from pure cultures of known fungi suspected of symbiotic relations. Both methods have been successfully used by Melin who thus obtained corroborative proof for the association of certain well-known Basidiomycetes with the roots of coniferous trees.

With regard to the technique adopted, it may be noted that satisfactory methods were devised for the external sterilisation of roots, and for the exclusion of fast-growing forms, e.g. species of *Penicillium*, *Mucor*, *Fusarium*, etc., likely to outgrow the true endophytes. For the detailed characters of the latter and their behaviour in pure cultures, reference must be made to the original records. Their essential features are indicated in the following brief summary.

(a) *The mycorrhizal fungi of the "Knollenmycorrhiza" of Pine.*
This type of mycorrhiza from many different stations yielded a number of fungus forms of similar type. Owing to the comparative ease of external sterilisation of the tubers and the relatively vigorous growth of the mycelia, their isolation was a comparatively simple matter. The characters and behaviour of all the strains in pure culture pointed to their inclusion in a single genus. Two morphological peculiarities may be noted: a characteristic paired branching of the mycelium in young cultures, and the invariable presence of

"clamp connections" (Schnallen) joining contiguous "cells." The first-named character appeared also in mycelium associated with the outside of the tubers and was later found to be characteristic of that of certain species of *Boletus* frequent in Pine woods; the last-named is characteristic of many, but not of all Hymenomycetes (Kniep, 1915); it served in this case for the classification of the endophytes into two groups characterised respectively by few or numerous 'clamp connections.' To the whole series of forms was given the name *Mycelium radialis silvestris* a. Later work supplied the necessary proof that the mycelium known by this name belonged to various species of *Boletus* abundant in Pine woods. Of these, *B. granulatus*, *B. variegatus*, *B. luteus* and *B. badius* have been identified with certainty as constituents of the tuberous type of Pine



Fig. 53. Aerial hyphae of *Mycelium radialis silvestris* a in pure culture. Strains isolated from trees in different parts of Sweden. (From Melin, 1925 a.)

mycorrhiza. *B. bovinus*, a common constituent of the fungus flora of Pine woods, may probably also be included.

The characteristic mode of branching exhibited by the mycelium of *M. r. silvestris* a in common with members of the genus *Boletus* is shown in Fig. 53. It is of interest to compare with this the mycelium figured by Rylands in 1842 as a constituent of the mycorrhiza of *Monotropa hypopitys* (see Fig. 2). The association of these forked hyphae with the roots of *Monotropa* may have been quite fortuitous; it may, on the other hand, be regarded as a possible clue to the identity of the root fungi of this species, in respect to which nothing is yet known.

(b) *The mycorrhizal fungi of the "Gabelmykorrhiza" of Pine.* Much greater difficulty was experienced in isolating the fungi responsible for this type of mycorrhiza, owing to their poor growth in artificial media. Three forms, differing in the morphological characters of their mycelia and believed to be representatives of a much larger group, were isolated and named respectively *Mycelium radialis silvestris* β , *Mycelium radialis silvestris* γ , and *Mycelium radialis silvestris* δ . The last-named was characterised by particularly feeble growth in artificial cultures. The mycelium of these strains showed Hymenomycete characters. Important differences in their structure and behaviour in pure culture rendered it unlikely that they belonged to a single genus or were nearly related as was the case with the forms included under *Mycelium radialis silvestris* α . Examination of the mycelium of various Hymenomycetes common in Pine woods provided no positive evidence of identity, although that of a number of species of *Tricholoma* and *Cortinarius* showed features resembling those in *M. r. s. \beta*, and *M. r. s. \gamma* respectively. No evidence was forthcoming for the identity of *M. r. s. \delta*. Further researches revealed the fact that several of the forms isolated could form mycorrhiza on the same tree; whether they might also be present in the same mycorrhiza was not ascertained.

In addition to the true root fungi, a form referred provisionally to the genus *Rhizoctonia* under the title *R. silvestris* was isolated from roots of young Pines which showed unsatisfactory growth. This fungus did not form typical mycorrhiza and was regarded by Melin as a parasite, sharply distinguished from the specific mycorrhiza formers.

(c) *The mycorrhizal fungi of Spruce.* Owing to their extremely feeble growth in pure culture, the mycorrhizal fungi of Spruce were likewise difficult to isolate, and only one form, *Mycelium radialis abietis*, was extracted and cultured. The mycelium showed Hymenomycete characters recalling those of *M. r. silvestris* γ from Pine, and it was believed to belong to a member of the same unidentified genus.

Final proof of the identity of the fungal forms just described with those responsible for forming mycorrhiza depended upon synthesis of the mycorrhizas under pure culture conditions. To this end, seedlings of Pine and Spruce were raised from sterilised seed and grown in small flasks in sand or humus cultures. The experimental seedlings developed normally without infection and showed certain structural features of interest in addition to the absence of mycelium, e.g. the absence of any marked differentiation to "long" and "short" roots, as well as a profuse development of root hairs from the main roots

and laterals. Inoculation experiments proved that each of the forms included under the names *Mycelium radialis silvestris* α , β , γ and δ from Pine, and *M. r. abietis* from Spruce, formed mycorrhiza. Owing to the fact that, even after leaching, sterilised humus is extremely toxic to these root fungi, sand, watered with a suitable nutrient solution, was found to be more favourable than humus for pure culture experiments, and it was only in such sand cultures that synthetic mycorrhizas were formed.

In general, the root fungi showed a marked increase in vigour when brought from pure culture into contact with the roots of ex-

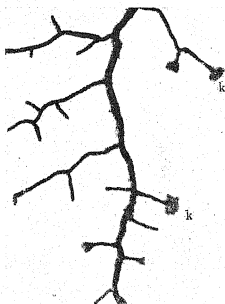


Fig. 54. Synthetic mycorrhizas of *Pinus montana* produced in pure culture with *Mycelium radialis silvestris* α . k. Knollenmycorrhiza. \times about $2\frac{1}{2}$ times. (From Melin, 1925 a.)

perimental seedlings. Although more vigorous than when growing alone, they were held to be less so than in nature, as evidenced by e.g. the absence of sporophores. Suggested causes for such decreased vigour were the absence of specific food substances normally present in natural humus, and the accumulation of the toxic by-products of growth in the culture flasks. The latter condition was known to have a depressing effect upon growth in pure cultures.

Synthetic mycorrhizas of two kinds were observed in these and similar cultures, some of the type described as pseudomycorrhiza with intracellular infection only, others of the *ectendotrophic* type described above. Some of the latter exactly resembled those found in nature, others lacked the mycelial investment or mantle; in

general, they were unbranched, but both the "Gabel"- and "Knollen"-mycorrhizal types were observed in artificial cultures. The details of structure, e.g. development of an intercellular "Hartig net" and the occurrence of intracellular infection with subsequent digestion, were similar to those in natural mycorrhizas (Fig. 54). It is noteworthy that artificial mycorrhizas of the pure ectotrophic type were not observed.

From comparative observations on Pine, Spruce and Larch, Melin subsequently reached the conclusion that the different types of mycorrhiza observed in nature represent phases in the development of infection, the typical ectotrophic condition being a final stage in a gradual "squeezing out" process due to enzyme activity in the root cells. On this view the endotrophic structure sometimes observed is transitional to the ectendotrophic condition, which in turn may give rise to true ectotrophic mycorrhiza with a thick mantle, well-developed intercellular net, and rare and intermittent intracellular infection. Fungal strains weakened by long-continued growth on artificial media or grown under unfavourable conditions, e.g. in sterilized humus or on a substratum of unsuitable H-ion concentration, can cause only endophytic infection, the exact nature of which will vary with the vigour or "virulence" of the strain used; the more vigorous strains can produce an intercellular net together with a well-developed external mycelial sheath or mantle. It was admitted that the absence of the ectotrophic type of structure from artificial cultures might be explained otherwise, for example, by assuming that the fungi responsible were more difficult to isolate, and that those actually extracted were the forms concerned in forming the ectendotrophic type, but it is clear that Melin himself inclines to the former view. The vigorous branching, so characteristic of natural mycorrhizas, was believed to be a direct consequence of the ectotrophic habit, and therefore, accepting the view just stated, to depend upon the presence of a fungus strain of relatively high "virulence." The higher the "virulence" the more marked the effect upon branching. Hence, under artificial conditions, one and the same fungus might give rise to *simple* mycorrhiza, "Gabelmycorrhiza," or "Knollenmycorrhiza" according to the degree of "virulence" induced by the conditions in respective cultures. The vigour or "virulence" of the individual strains was believed to be bound up with soil conditions nutritive and otherwise. This aspect of infection has since been more fully investigated and dealt with by the author in a separate communication (Melin, 1925). (See p. 367).

It has been shown by experiment that certain of the mycorrhiza-forming fungi are more specialised than others: some can form mycorrhiza in members of several genera, others only in those belonging to a single genus, or, in extreme cases, in only a few species of one genus. To the more specialised group belongs *Boletus elegans*, a mycorrhizal fungus of Larch (Melin, 1922), to the less specialised, other species of *Boletus*, e.g. *B. scaber* and *B. rufus*, mycorrhiza formers in Birch and Aspen. It is still unknown whether the forms associated with Pine and Spruce belong to the more specialised types. It was, however, observed in synthetic cultures, that not all those isolated could form mycorrhiza in a particular tree with the same ease, for example, in Spruce, *M. r. abietis*, *M. r. silvestris* β and *M. r. s. \gamma* are extremely active, while *M. r. s. \alpha* (*Boletus* spp.) have a relatively feeble capacity to form mycorrhiza, i.e. they are mycorrhiza formers of the second order. (See p. 367.)

In addition to the true mycorrhizal fungi, it has been ascertained by Melin that a large number of casual soil species can cause root infection of the pseudomycorrhizal type. Certain of these casual associates, e.g. *Penicillium* sp. (see Peklo, 1909), and *Acrostalagmus* sp. are harmless to the trees; others, e.g. *Mucor ramannianus* (Möller, 1903) and *Verticillium* sp. (Fuchs, 1911), behave as parasites. The mycelium of the fungus named *Mycelium radicis atrovirens* was frequently found to be present in old and young mycorrhizas, especially in the former, and proved comparatively easy to isolate. This mycelium formed sclerotia in culture and within the root cells, and it is believed that the species has affinities with the genus *Rhizoctonia*. Under pure culture conditions the fungus was purely parasitic and behaved as a pathogen; as observed by Melin, its presence in natural mycorrhizas is probably deleterious to growth and hinders the action of the true root fungi. It is possibly identical with the form in the mycorrhiza of Beech described by Peklo (1913) as:—"Kein Mykorrhizenbildner, sondern Parasit sei."

*The Experimental Identification of Certain Hymenomycetes
with the Mycorrhizal Fungi of Pine.*

In an earlier paper Melin (1922) had stated his conviction that the mycelium of the root fungi of Pine included under the name *Mycelium radicis silvestris* α belonged to the genus *Boletus*—" *M. r. silvestris* α gehört zur Gattung *Boletus*, umfasst aber wahrscheinlich mehrere Arten, von denen *B. luteus* eine ist." This belief, based on close resemblances in the mycelial characters and the frequent asso-

ciation of sporophores with the trees, was tested experimentally by the inoculation of pure culture seedlings from cultures isolated from the sporophores of various species of *Boletus* found growing naturally. The forms investigated, *B. luteus*, *B. variegatus*, *B. granulatus* and *B. badius*, are practically confined to coniferous woods and are specially characteristic of pure pine woods or mixed woods of Pine and Spruce. They were easily isolated and grew readily on malt agar and malt gelatine.

Synthetic cultures showed that all four species formed mycorrhizas in Pine. Under culture conditions only simple or dichotomously branched mycorrhizas (Gabelmycorrhiza) were formed, but it was believed that, under the more favourable conditions existing in natural humus, similar root infection led also to the formation of the tuberous type of mycorrhiza (Knollenmycorrhiza). In woods it is usual to find the latter strongly developed in the humus below fruit bodies of *Boletus* sp. Certain characteristic features of natural "Knollenmycorrhiza," e.g. the large-celled character of the mantle, were also noticeable in the synthetic mycorrhizas.

From analogy with Birch (Melin, 1924) it was regarded as likely that species of *Amanita* and *Tricholoma*, genera well-represented in Pine and Spruce woods, were also concerned in the formation of coniferous mycorrhiza. It may be recalled that the mycelium of the form *M. radicis silvestris* β , closely resembled that of species of *Tricholoma*.

B. THE MYCORRHIZA OF *Pinus montana*.

The conclusions reached by Müller (1903) in his historic investigation on the relation of the Mountain Pine to the growth of Spruce on the Jutland heaths (p. 338) rendered the root biology of the former tree of special interest to botanists and foresters. In order to ascertain whether the same mycorrhizal fungi were present as in *P. sylvestris*, Melin extended his investigation to include a series of pure culture experiments with seedlings of *P. montana*. They proved more amenable to culture under experimental conditions than had those of *P. sylvestris*, and the synthetic mycorrhizas developed in sand cultures more closely resembled those formed in nature. The production of pseudomycorrhizas in certain cases was attributed to the use of fungus cultures weakened by long cultivation, and consequently lacking the "virulence" necessary for the formation of the typical condition (Fig. 54). Using three of the fungus strains isolated from *P. sylvestris*, viz. *M. radicis silvestris* α , β , and γ , it was found

that all these forms likewise formed mycorrhiza in *P. montana*. In the *a* group (*Boletus* spp.), it was proved by direct experiment that *B. granulatus*, *B. luteus* and *B. variegatus* also formed mycorrhiza in Mountain Pine; that *B. badius* did not form typical mycorrhiza in the latter in synthetic cultures was attributed to the marked tendency shown by this species to degenerate under "pure culture" conditions.

A number of other Hymenomycetes common in Swedish woods were also tested experimentally, and it was determined that *Russula fragilis*, *Lactarius deliciosus*, *Cortinarius muscosus* and *Tricholoma virgata* all formed synthetic mycorrhizas in *P. montana*. Furthermore, it was regarded as probable that other species of these genera, and also *Amanita rubescens* would give similar results if tested in like manner. In general, it was concluded that the two species of Pine possess the same mycorrhizal fungi.

C. THE MYCORRHIZA OF LARCH.

Following his own observations and experiments, Melin (1922) reached the conclusion that an obligate mycorrhizal relation with the mycelium of *Boletus edulis* existed in Larch. Subsequently, Hammerlund (1923) reported that he was unable to obtain experimental confirmation of this and he rejected Melin's conclusions on the subject. In his most recent contribution to the literature of mycorrhiza, Melin (1925) has restated his former conclusions, citing *B. elegans* as an example of the most specialised type of mycorrhizal association, in which a given fungus forms mycorrhiza only with members of a single genus of vascular plants:—"Zu den spezialisiertesten gehört *Boletus elegans*, der ganz und gar an die Lärche gebunden zu sein scheint."

The statement that this species of *Boletus* is associated with trees other than Larch in southern Europe (Lange, 1923) is believed to require confirmation.

D. THE MYCORRHIZA OF BIRCH AND ASPEN.

In order to acquire a many-sided acquaintance with tree mycorrhiza, Melin made a study of Birch (*Betula pendula* Roth. and *B. alba* Roth.), and Aspen (*Populus tremula* L.) as representatives of deciduous trees (Melin, 1923). As in the case of coniferous mycorrhiza, there exists an extensive and contradictory literature on the subject; certain Hymenomycetes, regularly found in Birch woods, had been repeatedly named as mycorrhiza formers, but experimental

evidence of their identity was in every case lacking. Paulson (1923) has published an account of Birch mycorrhiza and described the infection of the styles of the ripe fruit by mycelium of a fungus believed to be *Sporotrichum pulviniforme*. The germinating seedling is exposed to infection by these hyphae, which, it is suggested, may be causally related to the subsequent formation of mycorrhiza. There is at present no experimental support whatever for the correctness of this interesting suggestion.

Melin's observations on Birch and Aspen have established the main facts experimentally, and have shown incidentally that the conflicting observations of earlier workers with regard to structure was due to faulty technique. The mycorrhizas of these trees are simply or monopodially branched, yellow or yellowish brown when young, becoming dark brown to black with age. They are of the ectendotrophic type with profuse intracellular infection. The strictly ectotrophic structure reported by Mangin (1910) and McDougall (1914) was not observed in Sweden, a discrepancy explicable either by non-formation of the latter under certain conditions of soil and climate, or by assuming a defective technique on the part of the earlier observers. A similar type of mycorrhiza is formed by both trees; since it differs in several respects from the coniferous type, that of Birch will be briefly described.

In transverse section the mycorrhiza of Birch shows a mycelial mantle of the usual ectotrophic type, a palisade layer of large, radially elongated cells with rich intracellular infection, and an inner zone several cell layers thick with profuse infection and typical intracellular digestion. The cells of the palisade layer are separated by a small-celled intercellular "net" of pseudoparenchyma, and contain hyphae of two kinds, described respectively as protein hyphae (*Eiweisshyphen*) and haustorial hyphae (*Haustorienhyphen*). The former are of large diameter (10 μ), with abundant protein contents and very large and conspicuous nuclei. They are branches from hyphae in the inner zone of the mantle which, traversing the palisade cells, may branch laterally, and eventually penetrate the cells of the digestive layer. The haustorial hyphae are of small diameter, with scanty contents and very small (*winzig*) nuclei; they may fragment in the palisade cells or penetrate the cells of the innermost zone where they undergo complete disintegration and digestion with subsequent disappearance of the stainable products (Fig. 55). As is usual in mycorrhizas, the endodermis and vascular cylinder is free from infection.

From structure alone, Melin has established a good case for the exchange of food material in these mycorrhizas with resulting benefit to the trees:—"Der anatomische Bau (des Birkes) zeigt dass die höhere Symbiont von den Pilzhypphen keineswegs geschädigt wird." The evidence for this, as for a similar condition in coniferous mycorrhiza, will be considered later. As well as the typical mycorrhizas, pseudo-mycorrhiza of the kind described for Pine is formed both by Birch and Aspen.

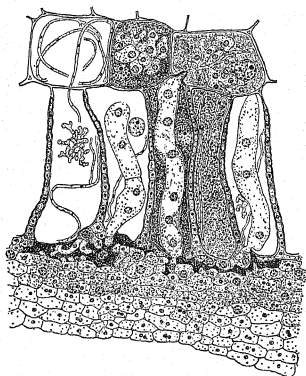


Fig. 55. Longitudinal section through a Birch mycorrhiza. Original drawing $\times 1000$. Reduced about $\frac{1}{3}$. (From Melin, 1923 a.)

As regards the identity of the root fungi, the commonest Hymenomycetes in northern Birch woods are *Boletus scaber* and *Boletus rufus*. The presence of these species is practically invariable but has been recorded also in other deciduous woods and exceptionally in coniferous woods. Thus Thesleff (1919) observed both species in Larch, Oak, and Alder woods; Peyronel (1917) reported *B. scaber* under *Fagus*, *Corylus*, *Castanea*, and *Sorbus aucuparia*. Moreover, Fries (1874) had noted the association of *B. scaber* with Birch and Köppen (1889) had recorded it from Birch woods throughout Russia.

In addition to these species of *Boletus*, a number of other Hymenomycetes are common in Birch woods and many had already been

cited as mycorrhiza formers. Woronin (1885) had named *B. scaber* and *B. edulis*; Frank (1892), *Amanita muscaria*; McDougall (1914), *Boletus* sp. and *Cortinarius* sp.; Peyronel, *Boletus scaber*, *Amanita muscaria*, *Lactarius necator* and *Scleroderma vulgare*. On account of their constant association with the respective trees, Smotlacha (1911) believed that *B. rufus* and *B. scaber* formed mycorrhiza in Aspen and Birch respectively.

Melin's researches were carried out by means of pure culture seedlings as described for Pine and Spruce, the ability to form synthetic mycorrhiza being tested and confirmed by inoculation from cultures isolated by appropriate methods from the sporophores of suspected Hymenomycetes.

Using similar methods, it was shown that many of the Hymenomycetes common in Birch woods are mycorrhiza formers. Decisive experimental proof has now been supplied by Melin for *Boletus scaber* and *B. rufus*, both of which formed mycorrhiza in Birch and Aspen, also for *Tricholoma flavobrunneum* and *Amanita muscaria* which formed mycorrhiza in Birch.

From previous work on Conifers it is regarded as probable that a number of other genera, notably species of *Lactarius*, *Cortinarius* and *Russula* are likewise responsible for mycorrhiza formation in Aspen and Birch. Moreover, although *Boletus edulis* did not form synthetic mycorrhiza under experimental conditions and appears only sporadically in Birch woods, it is probably a mycorrhiza former of the second order. *Boletus luteus* and *B. badius*, so characteristic of coniferous woods, were not observed to form mycorrhiza either in Birch or Aspen.

As was the case with Pine and Spruce, the experimental seedlings lacking infection grew freely, and showed similar modifications of the root system. It follows, therefore, that there is no obligate relation with the developmental seedling stages of these trees as in Orchids and Heaths.

Certain earlier observers, e.g. Brefeld (1908) and Duggar (1905) had noted the difficulty of cultivating the humus-dwelling Hymenomycetes on artificial media; the genera *Cortinarius*, *Lactarius*, *Amanita*, *Russula* and *Boletus* have all been named in this connection. These records, coupled with his own observations, led Melin to the view that such refractory species are possibly obligate mycorrhizal symbionts, incapable of growth, or growing with difficulty,

in the absence of certain nutritive materials ordinarily obtained from the roots of their host trees.

The list of Hymenomycetes experimentally identified as mycorrhiza formers in Pine and Spruce has since been extended to include *Amanita muscaria*, *Cortinarius muscosus*, *Lactarius deliciosus* and *Russula fragilis* on Pine, and *Amanita muscaria*, *Cortinarius balteatus*, and *Lactarius deliciosus* in Spruce (Melin, 1925). No proof has yet been obtained as to the identity of the forms known respectively as *Mycelium radicans silvestris* β , γ , δ , or of *Mycelium r. abietis*.

Fresh light has been thrown upon the physiological aspects of mycorrhiza in forest trees in an experimental research designed by Melin (1925) to elucidate the physiology of nutrition in both symbionts and test the nature of their mutual relations. The tentative conclusions reached by the author himself are clear from his earlier papers. Thus:—"Der anatomische Bau des beschriebenen Mykorrhizatypus spricht also für einen gegenseitigen Stoffwechsel zwischen den beiden Konstituenten, d.h. dafür, dass wir es mit einer wirklichen mutualistischen Symbiose zu tun haben" (Melin, 1923 a, p. 94), and again, in relation to the mycorrhiza of Birch and Aspen:—"Die Birken- und Espenmykorrhiza ist kein parasitisches Gebilde, wie es z.B. McDougall meint, sondern Pilz und Wurzel leben in mutualistischer Symbiose miteinander" (Melin, 1923 a, p. 517).

These opinions survived the test of experiment. Valuable results were obtained from an experimental study of the root fungi of Pine and Spruce in pure culture, and the conclusions based upon them constitute an important contribution towards the solution of a much-discussed and highly controversial part of the mycorrhiza problem. Two aspects of this work demand attention: one, the nutritive reactions shown by the symbionts in pure culture with special reference to the assimilation of nitrogen; the other, the application of the experimental results to conditions found in nature.

As compared with indifferent soil species, the growth of the mycorrhizal fungi of Pine and Spruce is variable and often very slow. Some species, e.g. the *Boleti* associated with these two trees, are relatively vigorous, others, e.g. *M. radicans silvestris* β , *M. r. silvestris* γ , and *M. r. abietis*, grow extremely slowly, others again, e.g. *Russula fragilis* and *Amanita muscaria*, are maintained in culture with difficulty. Some species form sporophores in artificial media, others do not. Many show loss of vigour due to cultural conditions, and the physiological change thus suffered is maintained when

the mycelium is subcultured to a favourable medium. Such inconsistencies of behaviour can be explained by the assumption that many of these fungi are obligate symbionts in greater or less degree, incapable of normal growth in the absence of their respective host trees. If this assumption be correct, the existence of such forms obviously limits the field of experimental enquiry.

Moreover, the mycorrhizal forms have been shown to be extremely sensitive to the H-ion concentration of the substratum. Whereas other soil fungi, e.g. *Rhizoctonia silvestris* and *Mycelium radialis atrovirens*, grow indifferently on acid and neutral media over a wide range of pH values, the true root fungi, with few exceptions, prefer acid substrata, grow badly on those with a neutral reaction and are incapable of growth at pH values on the alkaline side of neutrality. Optimum conditions for the fungi of Pine and Fir are provided by pH values between 4.0 and 5.0. The correctness of these experimental values receives support from Hesselman's (1917) field observations recording a pH value of about 4.0 in the humus layer of coniferous woods throughout northern and middle Europe. In Finland likewise, Brenner (1914) reported values from 3.5 to 4.8 for similar soils. Isolated observations on the H-ion concentration in root cells, e.g. those of Arrhenius (1922) recording a pH value of 4.5 for the root cells of Wheat have a possible significance in the same connection.

The reaction of the root fungi to small amounts of phosphatids is of particular interest in view of Hansteen-Cranner's observation that, under certain conditions, roots of the higher plants excrete phosphatids, not only when immersed in distilled water but also in nutrient salt solutions and fertile soil. (Hansteen-Cranner, 1922.) Furthermore, it is known that the growth of many species of bacteria is stimulated by root excretions (Wilson, 1921), (Berthel, 1923), and this fact, combined with his own observation that contact with roots of the appropriate host trees perceptibly stimulated the growth of mycorrhizal fungi, led Melin to examine the reaction of the latter to substances excreted from seeds and seedlings of Pine and Spruce¹.

The results obtained from cultures for which different nutrients and combinations of nutrients were used, indicated that the diffusible substances caused a marked stimulation of growth. For example, after 40 days in contact with seeds or seedlings, *Boletus variegatus* gave a dry weight value sixteen times as great, and *M. radialis*

¹ It had been stated previously (Hansteen-Cranner, 1922) that living seeds and seedlings of these trees give off appreciable amounts of (water soluble) phosphatids at 20° C.

abietis one fifty-six times as great as when grown alone. Control cultures in which dead seeds were substituted showed no effect of this kind. Similar results were obtained with ordinary soil fungi. Assuming that the concentration of phosphatids is proportional to the number of seeds used, the experiments indicate that an optimal concentration is quickly reached, after which no increase in growth can be observed. It was concluded by Melin that the effect is similar in kind to that brought about in *Penicillium* by the addition of an extract of Yeast (Lepeschin, 1924).

Experiments to test the reaction of the fungi of Pine to various nitrogenous food materials proved that none of the forms experimented with could utilise atmospheric nitrogen; salts of ammonia, urea, and nucleic acid were all used as sources of nitrogen, while individual fungi could equally well utilise peptone, asparagin and a number of organic compounds. Assuming enzyme activity in the mycelium to be comparable with that found in the sporophore, Melin's conclusion that mycorrhizal fungi in nature can utilise different organic compounds or groups of such compounds in humus soils may be safely accepted.

Variation in the nature of the carbon compounds supplied to cultures showed that growth was satisfactory only when glucose was added, thus providing confirmation of the popular view that mycorrhizal fungi obtain carbon compounds from the root cells. Direct observations on humus were difficult owing to the toxicity produced by heat sterilisation. Chemical methods of sterilisation were not regarded as satisfactory, but humus extracts freed from micro-organisms by filtering gave weak growth, the addition of glucose producing vigorous development as in a favourable soil.

The reaction of uninfected seedlings of Pine and Spruce to various nutrients has also been experimentally tested. As might be expected, no evidence was found that seedlings in pure culture could fix atmospheric nitrogen. Inorganic compounds of nitrogen, e.g. potassium nitrate and ammonium chloride, proved to be favourable sources of nitrogen; simple organic compounds, e.g. asparagin, were also readily utilised, but more complex bodies like peptone and nucleic acid were assimilated with difficulty, and seedlings supplied with these substances soon showed symptoms of nitrogen starvation (Fig. 56).

From the experimental results yielded by seedlings and root fungi grown separately under pure culture conditions it was unlikely that synthetic cultures would exhibit any capacity to utilise

atmospheric nitrogen. In view of its great importance this possibility was carefully tested and gave negative results, the small increase in nitrogen content in seedlings grown in substrata lacking combined nitrogen being regarded as due to impurities in the air of the laboratory. Melin has found no evidence of nitrogen fixation on the part of any coniferous tree and the claim put forward by Müller in respect to *Pinus montana* has received no experimental support.

The root development observed in experimental seedlings varied directly with the nature of the nitrogen supply, e.g. roots supplied with nucleic acid were from four to six times as long as those supplied with potassium nitrate, ammonium chloride or asparagin.

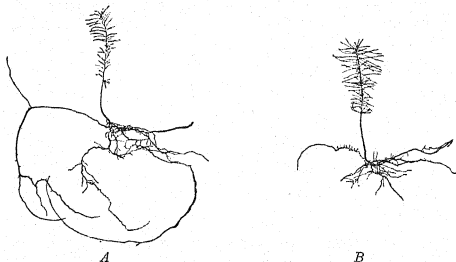


Fig. 56. Seedlings of *Picea abies*, three years old, in pure culture, with nucleic acid (Nukleinsäure) supplied as a source of nitrogen. A, seedling without fungus infection; B, seedling infected with the mycorrhizal fungus, *Mycelium radialis abietis*. About $\frac{1}{4}$ natural size. (From Melin, 1925.)

The experiments made by Melin to test the reaction of infected as compared with uninfected seedlings towards various compounds of nitrogen are of special interest and importance in view of the historic controversy as to the nature of nutrition in tree mycorrhiza and its relation to the soil humus. The comparative growth of synthetic cultures as compared with that of pure culture seedlings supplied in each case with ammonium chloride, peptone or nucleic acid was measured by means of dry weight estimations. In general, the figures obtained and the condition of the cultures justify Melin's conclusion that the mycorrhizal fungi facilitate the intake of ammonium salts and other organic compounds by the tree, although it is not clear that such intake may not be carried out more rapidly under pure culture conditions by roots free from fungus infection.

By supplying more complex compounds, e.g. peptone or nucleic acid, evidence was obtained that these could be assimilated more readily by mycorrhiza than by uninfected roots, thus permitting the conclusion that mycorrhizas are organs directly related to the nutrition of the tree and having a special importance in relation to the assimilation of organic nitrogen. For example, for a number of three-year-old Pine seedlings, the average percentage nitrogen content was 2.86 in seedlings with mycorrhiza, as compared with 2.64 in those lacking infection. The prevalence and robust development of mycorrhiza in raw humus soils thus becomes extremely significant (Fig. 56).

In order to test the Stahl hypothesis in respect to inorganic salts, experiments were carried out with different concentrations and combinations of salts, final conclusions being based on a comparative study of the ash content of 50 plants. The values obtained (Melin, 1925, Table 43) pointed to the conclusion that inorganic mineral salts can be absorbed with equal facility by infected and uninfected roots in pure culture. On the whole the experimental results were in agreement with the Stahl hypothesis in respect to mineral salts, although the benefit derived by the trees was regarded by Melin as quite secondary to that associated with nitrogen assimilation.

With regard to the maintenance of a "balance of power" between the mycorrhizal fungi and their hosts, it is clear from the pure culture experiments described that the development of normal mycorrhiza is bound up with, and directly depends upon, the physiological state existing in each of the two symbionts, the health and vigour of the seedlings on the one hand, and the condition of the mycelium on the other hand, each playing an important rôle. The ectotrophic and endotrophic types of structure may be regarded as independent reaction products of the joint activities of mycelium and roots. Given an unhealthy condition of the latter and mycelium of high "virulence," the relation may degenerate into one-sided parasitism with the fungus in command of the situation. Assuming healthy root condition, the development of normal mycorrhiza depends upon those physiological qualities in the mycelium which constitute "virulence." In respect to this, Melin has proposed a classification of the root fungi studied into three classes described as *active*, *less active*, and *inactive* respectively. In relation to a particular host, members of the first class form typical mycorrhiza more or less easily under normal conditions; they are mycorrhiza builders of the first order. The fungi included in the second class produce mycorrhiza slowly and with difficulty; they are mycorrhiza builders of the second

order. Those belonging to the third group are unable to form either ectotrophic or endotrophic mycorrhiza. In each class are included forms of slight, medium, and relatively high "virulence," as evidenced by their behaviour when brought into contact with seedling roots, their reaction in each case being determined both by the inherent specific qualities of the mycelium and by fluctuating changes in the environment. There is at present no full explanation of the observed fact that one and the same fungus may produce *intracellular* infection, and, under other conditions, give rise to an *intercellular* net without penetration of the cells.

Melin has correlated the failure to form mycorrhiza of the ectotrophic and endotrophic types in artificial cultures with decreased "virulence" on the part of the constituent root fungi. Seeking a cause for the latter condition, he determined experimentally that the true mycorrhiza formers grew feebly in substrata of unsuitable H-ion concentration, and finds in this fact an explanation both of his own experimental observations and of the distribution of coniferous mycorrhizas in nature. In view of the evidence offered, there can be little doubt that the reaction of the substratum is of great importance in determining the structural character of the mycorrhizal association. (Melin, 1924 *b*.) These observations have rendered it possible to attach a more precise physiological meaning to the varying degrees of "virulence" postulated by Melin. Exactly in what manner the biological relation is affected by the different types of structure observed in coniferous mycorrhizas is at present unknown.

No evidence has been obtained for the existence of different strains or *genotypes* of the same fungus species in association with different trees. For example, under cultural conditions one and the same strain of *Amanita muscaria* formed mycorrhiza in Pine, Spruce, Larch and Birch; one and the same strain of *M. r. silvestris* formed it in Pine, Spruce and Birch.

The application of these results to natural conditions is of great practical interest. In view of the experimental observations, factors likely to be effective are:—(1) the H-ion concentration of the soil, (2) the condition of the organic constituents of the soil, (3) the presence in soil of substances which inhibit growth of the root fungi. With regard to the first of the factors, Hesselman (1917) and others have reported pH values of 4.0 to 5.0 in coniferous humus throughout Sweden, and in such acid soils (*Rohhumusböden*) mycorrhiza is freely produced. In deciduous woodlands Hesselman and Arrhenius (1920)

found pH values between 6.0 and 7.0. In these neutral and slightly acid soils (*Mullböden*) Pine and Spruce form mycorrhiza only sporadically, although the absorbing roots frequently develop pseudomycorrhiza as a result of invasion by relatively inactive forms or by indifferent soil fungi. The variable development of mycorrhiza observed in different types of acid soils may be related directly to the second set of factors named above, namely, the presence of organic compounds of nitrogen acceptable to the mycorrhizal fungi, the observed concentration of mycorrhiza in the upper layers of humus being possibly due to the same cause. It is not without significance that the true root fungi show greater sensitiveness in this respect than do *Rhizoctonia silvestris*, *M. r. atrovirens* and casual soil fungi generally.

With regard to the existence in natural woodland soils of substances deleterious to the growth of mycorrhizal fungi there is at present no information. Under experimental conditions these forms are extremely sensitive to the accumulated by-products of growth in the substratum, and mycelium from such poisoned cultures does not build mycorrhiza. Viewed in the light of modern work upon soil conditions, the experimental results point to the conclusion that Conifers are not strictly autotrophic in respect to their nitrogen metabolism when growing on humus soils. In Melin's words:—"Die Mykorrhizen sind auf Rohhumusböden sehr günstige stickstoffvermittelnde Organe, da die Pilzsymbiont ebenso wie die anderen Bodenpilze leicht Ammoniak und organische N-Verbindungen assimilieren können." If this conclusion be correct, trees possessing typical mycorrhiza are remarkably well equipped for competition with members of the soil microflora under the conditions stated.

It is more doubtful whether the mycotrophic habit is indispensable to the healthy growth of Conifers on neutral humus soils. On the other hand, well marked differences are known to exist in such soils in respect to the rate at which nitrification takes place, and the conditions responsible for such differences may be causally related to an irregular development of mycorrhiza.

Passing now to the effect of mycorrhiza formation upon the constituent fungi, it may be noted that the stimulating effect produced by contact with the host tree appears to be related definitely to the excretion of phosphatids. It has been suggested by Melin that certain of the mycorrhizal fungi may be unable to complete their development, e.g. form fruit bodies, lacking this stimulus. This assumption requires experimental verification. If correct, it provides

a satisfactory explanation of the observed disappearance of Hymenomyces from cleared woodlands. In the evolutionary sense, the fungi concerned in mycorrhiza formation may probably be regarded as members of the saprophytic soil flora which have become adapted to symbiotic existence, although, in certain cases, e.g. *Tricholoma* sp., they remain relatively independent of it. Assuming the correctness of Melin's general conclusion:—i.e. "dass die Mykorrhizen für die Pflänzchen und Bäume auf Rohhumusböden und verwandten Bodentypen eine vitale Bedeutung besitzen," the application of the new knowledge gained from experiment becomes a matter of great practical interest, and opens up a fascinating new field of research in relation to forestry.

His work has confirmed the view certain to be reached by every modern student of mycorrhiza; namely, that there is no sharp distinction of the kind postulated by Frank between the ectotrophic and endotrophic types of structure. The formation of an external sheath and the intercellular development known historically as the "Hartig net" do not preclude the existence of an intracellular distribution of the same mycelium. Recent experiments have shown that the type of mycorrhiza formed is controlled, in the case of certain conifers, by the physiological condition of the endophyte and by the rooting condition of the host. It is, therefore, a resultant of these conditions and must be regarded as an expression of the physiological activities of the two symbionts at the time of observation. The factors that influence these activities are still relatively little known; their study offers a profitable field of enquiry and one which must be more fully explored if there is to be any real understanding of the nutritive relations between fungus and vascular plant in the case of trees.

Certain recent researches on the decomposition of woodland humus point to conclusions significant in this connection.

In humus soils the liberation of plant food from the organic constituents is known to depend upon chemical changes brought about by the nutritive processes of members of the soil microflora and microfauna. Since these processes involve complex chemical changes, the subject is extremely intricate; it is also of great practical importance in relation to forestry. Although not necessarily or immediately concerned with mycorrhiza, it is closely bound up with the activities of soil fungi, and hence comes into touch with the ecological and physiological aspects of root infection.

The deterioration of forest soils resulting from an abnormal accumulation of raw humus is stated by Falck (1924) to be directly related to the absence of certain members of the Higher Fungi. In fertile forest soils, certain members of the Higher Fungi, especially Hymenomycetes and other Basidiomycetes, with members of the Mucoraceae, are believed to be primarily responsible for the complete decomposition of fragments of wood, leaves and other plant residues. Falck has investigated and described the chemical changes involved in this process, to which he has given the name "Mykokrinie." His researches show likewise that the normal process of decomposition by fungi is often interrupted by the activities of insect larvae, which devour both the mycelium and the detritus on which it is growing, converting it eventually to a dark-coloured crumbling humus mass—a process described under the name of "Anthrakrinie." In humus soils undergoing this kind of decomposition four layers may be distinguished:—

- (1) An upper layer of unchanged plant detritus;
- (2) A region in which mycelial activity is conspicuous, especially in spring and early summer;
- (3) The humus layer proper, composed below chiefly of dark-coloured insect excreta and passing upwards into partially decomposed vegetable detritus;
- (4) The uppermost layer of mineral soil containing the soluble material from the humus layer above leached out by rain, and traversed by a dense network of tree roots.

The deterioration of forest soils, due to an abnormal accumulation of raw humus and consequent deficiency of available nutrients, is believed to be due mainly to the absence of the ordinary woodland type of fungus flora. Failing the activities of suitable fungi, leaves and other residues collect upon the surface and undergo chemical changes of an entirely different kind, resulting in the conversion of the raw humus to "dry peat" (Trockentorf) in which the carbon, nitrogen, phosphorus, and potassium are locked up and rendered permanently inaccessible to the trees. This formation of peat or "Vertorfung" is named "Anthragenie," and can be related by transitional types of change to the complete decomposition taking place in *mycocriny* and *anthracriny*.

The factors promoting the latter are believed to be the following: high atmospheric humidity associated with high soil temperature; a sufficiency of lime; an abundant and suitable fungus flora; the favourable character of the detritus.

The relatively satisfactory development of many kinds of trees on dry peat or other humus soils in which no progressive decomposition is taking place is explained by an appeal to the mycotrophic habit, i.e. by the assimilation of organic nutriment from humus-forming detritus with the help of mycorrhizal fungi.

The benefits accruing to forest trees from symbiotic relations with mycorrhizal fungi—excluding nodule-forming associations, in which nitrogen fixation occurs—consist, in Falck's opinion, solely in the presentation of the requisite foodstuffs in the form of organic solutions.

The advantages of *mycocriny*, *anthracriny* and the mycotrophic habit as compared with *anthrageny*, in relation to practical problems of forestry, are discussed by Falck, and he points out that trees with well-developed mycorrhiza are far better adapted to unfavourable climatic and soil conditions than are those devoid of fungus symbionts. Suggestions are made also for promoting the decomposition of humus by biological methods.

Melin has reported that soil Hyphomycetes do not form mycorrhiza in trees, although certain members of the group, e.g. species of *Rhizoctonia*, are known to invade roots more or less parasitically and to give rise to pseudomycorrhiza. Viewed in the light of Falck's observations on the distribution of fungi in forest soils, this conclusion is not without interest.

The ectotrophic habit is typically associated with trees and shrubs. For this reason, in the present chapter, attention has been focussed upon the mycorrhiza of trees, more especially upon those recent experimental researches from which alone can be gained any knowledge as to the real significance of the habit. A few isolated cases of this type of mycorrhiza are known in herbaceous plants, notably the classical one of *Monotropa* and its allies. Most of these have received mention elsewhere in the present work; in none of them has the biological character of the relation yet been illuminated by experimental enquiry.

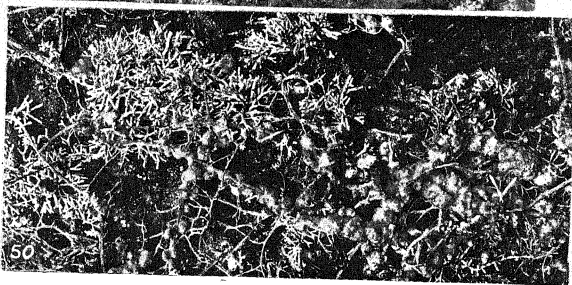
(To be continued)

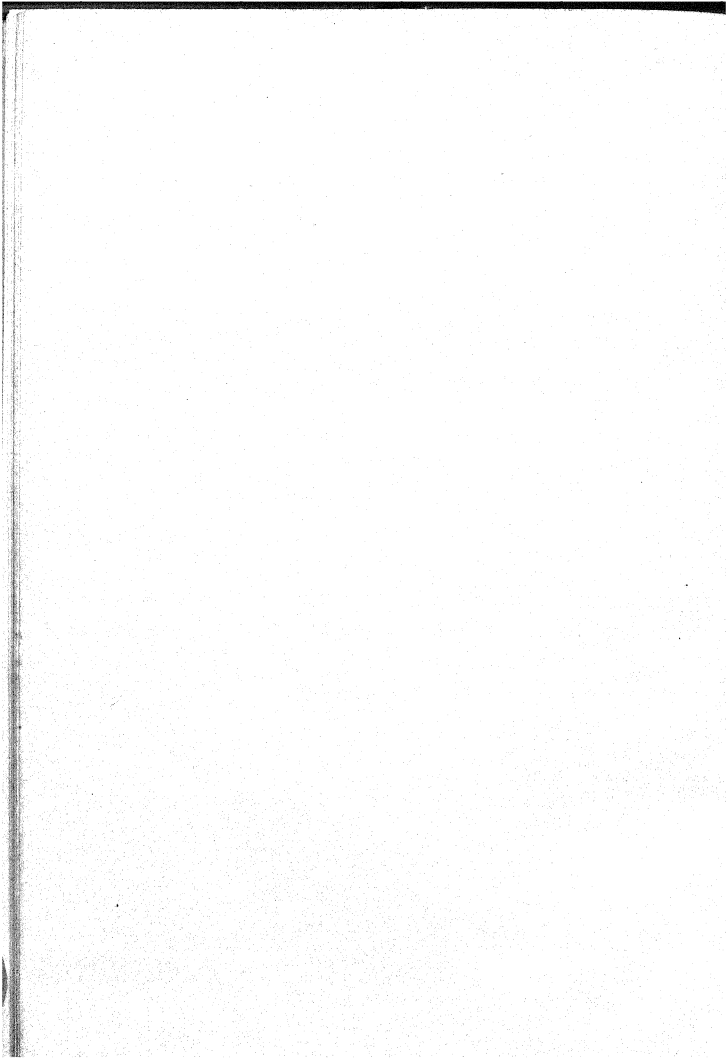
EXPLANATION OF PLATE VIII

Fig. 50. "Knollenmycorrhiza" and "Gabelmycorrhiza" borne on the same main root of Pine (*P. sylvestris*). (From Melin, 1923.)

Fig. 51. Mycorrhiza of Beech in superficial deposits of fallen leaves below the trees.

Fig. 52. "Fairy ring" of *Clitocybe* sp. under Larch, Co. Wicklow, Ireland.





NEW DISCOVERIES IN THE MIDDLE DEVONIAN FLORA OF GERMANY

By D. H. SCOTT, F.R.S.

IN 1923 Drs Kräusel and Weyland published a paper on the Devonian Flora of the neighbourhood of Elberfeld¹. This work, though containing much of interest, was to some extent a preliminary notice; since then important new researches have been carried out, and the authors' second paper, published this summer², is the most considerable contribution to Devonian palaeobotany since the Rhynie discoveries. It is on this second paper that the following remarks are based.

The specimens came from several localities near Elberfeld. Use was made of various public and private collections especially those of Herr Piedboeuf, who gave much help. The new finds seem to have been chiefly made in the quarry at Kirberg. The horizon is that of the "Honseler Schichten," belonging to the lowest division of the upper Middle Devonian.

It is a fortunate circumstance that the plant remains, while showing the external habit, are often partly petrified, allowing of anatomical investigation. Owing to the nature of this petrification no thin sections could be made; polished surfaces were therefore examined, in reflected light, with good results.

Five plants are described. Others still remain to be investigated. The plants dealt with are: *Asteroxylon elberfeldense* n. sp., *Aneurophyton germanicum* K. and W., *Hyenia elegans* n. sp., *Calamophyton primaevum* n. gen. et sp., *Cladoxylon scoparium* n. sp.

The *Asteroxylon* is of great interest, for comparison with the now well-known Rhynie species. Branching, naked axes, formerly referred to *Hostimella*, are extremely common in the deposit. The ends of the shoots are sometimes circinate. In two cases a sporangium was found terminating a branch. It is now shown that these "*Hostimella*" branches pass over below into spiny stems, of the *Psilophyton* type. Still lower down, the thorny axes are continuous with stems bearing

¹ R. Kräusel and H. Weyland, "Beiträge z. Kenntnis der Devonflora," *Senckenbergiana*, Bd. 5, Hefte v, vi. Frankfurt, 1923.

² "Beiträge z. Kenntnis der Devonflora, II," *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft*, Bd. 40, Heft II. Frankfurt, 1926. With 15 Plates and 46 Text-figures.

scale-leaves, identical with *Thursophyton*. Thus the Elberfeld plant combines in itself the three so-called genera *Hostimella*, *Psilophyton* and *Thursophyton*. An affinity with the two last was already detected by Kidston and Lang, but the identity shown in the German plant goes beyond what was expected. The *Hostimella* branches are believed to correspond to Kidston and Lang's sporangiophores, found in association but not in connection with the Rhynie *Asteroxylon*.

The *Hostimella* region may follow almost immediately on the leafy *Thursophyton* stem, and may probably, in exceptional cases, even spring directly from the rhizome. But the longer the whole plant is, the more clearly are the successive regions differentiated.

As regards the anatomy, the stele is round in section in the *Hostimella* portion, but sometimes shows an approach to the stellate form, which is well developed in the lower regions of the stem. Even in the thicker parts of the rhizome a stellate stele may occur. Unlike *A. Mackiei*, a pith is present in the stellate stele. No bundles were observed to pass out into the leaves. The tracheides of the wood are usually scalariform but may sometimes have round pits. In their earlier paper Kräusel and Weyland mentioned secondary thickening as occurring, but this idea has turned out to be fallacious.

There is evidently a far-reaching agreement between the Elberfeld and the Rhynie *Asteroxylon*. They differ, however, in the presence of a pith in *A. elberfeldense*, in the assumption of the stellate form of stele even in the leafless regions and in the occurrence of pitted tracheides. No stomata were observed in the German species, but this was probably a matter of preservation. The discovery of the *Hostimella* region in *A. elberfeldense* establishes a stronger probability than before that the similar sporangiophores associated with *A. Mackiei* really belonged to the plant.

Both *Hostimella* and *Thursophyton*, and we might add *Psilophyton*, are heterogeneous groups, which may well include forms quite distinct from those which have been shown to form part of *Asteroxylon*.

As regards the habit of *A. elberfeldense*, the authors consider that it was a water or bog plant. Specimens 60 cm. long have been found and it is probable that a total height of as much as a metre may have been attained¹.

¹ By kind permission of the authors a very brief notice of *Asteroxylon elberfeldense* was published by the present writer in *Conquest* for March 1926, p. 102, and illustrated by a reduced copy of their restoration of the plant.

The next plant, *Aneurophyton germanicum*, was already described in the authors' first paper, but a more complete account is now given. *Aneurophyton* is regarded as probably a tree, with the habit of a tree-fern. The fronds are large and highly compound; a portion figured was over a foot long. The word "frond," however, is used in a merely descriptive sense; the anatomy shows that the rachis had the structure of an axis; only the ultimate pinnules appear to have been of the nature of true leaves; they are nerveless—hence the generic name. The plant thus seems likely to have an important bearing on the question of the origin of the fern-like frond.

Sterile and fertile fronds are distinguished. The latter bore the sporangia on special pinnules, incurved when young. The sporangia first described are elongated and shortly stalked, without an annulus. A possible second form with the sporangia in tufts, more like a *Telangium*, has since been found, but its relation to the plant is doubtful.

The anatomical structure has been observed in various parts, from pieces of stem about 2 cm. thick down to branches of the rachis. It is always essentially the same. The wood consists of radially seriate tracheides interspersed with medullary rays one or two cells wide. The tracheides are either scalariform (probably in the inner zones of the wood) or reticulate. Bordered pits may have been present. The pitting is on all the walls alike, an important point of agreement with *Palaeopitys Milleri*, as investigated by Kidston and Lang. The centre, where the primary wood may have been, had always perished. Root-like appendages were also examined anatomically; their secondary wood is like that of the stem.

Kräusel and Weyland compare their plant closely with the American *Eospermatopteris*, from the fossil forest of Gilboa, of similar geological age. In the habit of the vegetative parts there is a complete agreement between the two fossils. There is, however, no evidence of seeds in the German plant, and the systematic position remains an open question.

Anatomically there is much in common between *Aneurophyton* and *Palaeopitys Milleri*, but the habit of the latter is unknown. The authors suggest the possibility that Lang's *Hostimella pinnata*¹ may have been an *Aneurophyton*. In their reconstruction of *A. germanicum* the authors have been guided by Miss Goldring's restoration of *Eospermatopteris*.

¹ W. H. Lang, "Contributions to the Study of the Old Red Sandstone Flora of Scotland," Parts I and II, *Royal Society of Edinburgh, Trans.* 1925.

The next two fossils belong to quite a different group, for they both show some affinity to the Articulatae. *Hyenia elegans* is first described; this species is in general similar to *H. sphenophylloides* Nathorst, from the Middle Devonian of West Norway. In *H. elegans*, however, the leaves are more repeatedly forked than in Nathorst's plant, while the number of leaves in a whorl is more probably three than four. In neither species is the stem jointed. The shoots reach a length of 16 cm., and a thickness of 4 mm.; they converge below, as if towards a rhizome or an upright stock; the authors think the latter more probable.

The point of chief interest in *H. elegans* is the presence of the fructification, previously unknown in the genus. It is in the form of loose spikes, the axis bearing numerous forked sporangiophores, but no bracts. Two or three pendulous sporangia are borne on each arm of the bifurcate sporangiophore. This species and *Calamophyton primaevum*, the next to be described, are the oldest plants known, of the Articulate type, in which the reproductive organs are preserved.

The affinities of *Hyenia* are best considered in connection with the succeeding species.

In this plant, named *Calamophyton primaevum*, the main stems are thick and apparently woody. They are freely branched by dichotomy, a most unusual feature in the Articulate group. On the younger branches there are clear articulations which become obscured on the older stems. The leaves on the small twigs are in definite whorls; they are small and delicate (about 10 mm. long) and forked once or twice. On the older stems the leaves are replaced by thorny excrescences regarded as modified leaf-bases. Strange to say, they are here spirally arranged. The authors attribute the displacement to secondary growth, but this explanation hardly seems adequate.

The fructification, as in *Hyenia*, forms loose cones, with a somewhat stout axis. Here also the whorled sporangiophores are forked, but so far as observed, only one sporangium was borne on each arm of the fork. Still, the similarity to *Hyenia* is very striking; here also no bracts are present.

The anatomy could be investigated in petrified parts of the stem. The stele is triangular in transverse section and possesses a pith, thus combining Sphenophyllian and Calamarian characters. The tracheides are scalariform. No secondary thickening was observed, but it probably occurred in older parts of the stem.

The authors compare both *Calamophyton* and *Hyenia* with the various families of Articulatae, and come to the conclusion that they

fit into none of them. They propose a new class, Proto-articulatae, consisting of the two families Hyeniaceae and Calamophytaceae, each of which represents a series of its own. To these are opposed the true Articulatae divided into the four series: Sphenophyllales, Cheirostrobales, Pseudoborniales and Equisetales (the last, of course, including Calamariaceae).

Considering the close agreement in the fructification between *Hyenia* and the new genus *Calamophyton*, one may doubt whether they should not be included in one series.

The last species described, *Cladoxylon scoparium*, is in some ways the most interesting of all the authors' discoveries, for it belongs to a genus which no one would have expected to find in the Middle Devonian. *Cladoxylon* has been a highly problematic group, ever since its first discovery by Unger in 1856. The extraordinary anatomy is well known, but hitherto there has been nothing to show the external habit of these plants. This is now for the first time revealed in Kräusel and Weyland's species, which further shows something of the fructification, equally unknown until now.

The material consisted of a single large specimen from the Kirberg quarry, and a fragment in Herr Piedboeuf's collection. The main specimen is made up of a number of stems, reaching 22 cm. in height, and converging downwards so as to suggest their springing from a common axis. The stems are sparingly branched in a dichotomous manner; the largest are about 1.5 cm. in breadth.

Leaves occur on the thicker as well as on the more slender parts; on the former they are small (5-10 mm. long), deeply cut and forked, often with a broad base. In the higher regions the foliage is denser, and the leaves larger (up to 18 mm. in length). They here have a distinct petiole, and are more finely divided. The phyllotaxis is spiral. The leaves must have been delicate and caducous—detached leaves are crowded round the finer branches. This is the first time that the foliage of a *Cladoxylon* has been observed.

Some of the branches bear leaves of a different kind—the sporophylls. They are fan-shaped, with a corresponding venation, while in the vegetative leaves no veins are shown. The anterior edge is deeply lobed; each lobe is expanded and hollowed at the end, like a spoon. The hollows of course appear convex on the opposite surface, and suggest the presence of small, round marginal sporangia. This suggestion is confirmed by the occurrence, close to the leaf-margin, of the impressions of sporangia (about 0.5 mm. in diameter). A

reticulate marking can be distinguished on the wall and numerous small isolated spores are present. No other form of fructification was found, and it is of course impossible to determine whether the bodies observed were ordinary spores or of the nature of microspores or pollen-grains.

So far the characters described are entirely novel, and would not by themselves enable us to identify the plant. It is the anatomy which proves it to have been of the *Cladoxylon* type. The petrification of parts of the stems allowed of the observation of the structure at various points. The vascular system is highly complex, consisting of a number of narrow steles more or less radially directed, much as in Unger's well-known species *C. mirabile*. A series of transverse sections (or rather surfaces) is figured, cut at intervals of 5 mm. from a branch about 2-3 mm. in thickness. At the top the structure is comparatively simple; it increases in complexity downwards, the steles becoming more numerous and so crowded at the lowest level reached as to form a dense woody tangle, even exceeding in complexity the species already known. The authors speak of the "polystely" as increasing "with age" ("mit dem Alter," pp. 146, 147). It is difficult, however, to understand how new steles could have been intercalated without secondary growth, of which no evidence was found. It may perhaps be conjectured that the branches examined were of limited growth, with a permanent range of complexity from base to apex.

In rare cases a round bundle, perhaps a leaf-trace, was observed passing out. The tracheides are scalariform or pitted; bordered pits are seldom demonstrable. No secondary growth was observed, but it may probably have taken place, as in other *Cladoxylons*, in thicker parts of the stem. The anatomical characters are sufficient to justify the reference of the plant to the genus *Cladoxylon*.

The small, sharply defined leaves refute the idea, once entertained on anatomical grounds, of an imperfect differentiation between stem and leaf in this group. *Cladoxylon* was evidently a very highly organised genus—whether a Pteridosperm or a Pteridophyte of some long extinct race remains undetermined.

The discovery of this Middle Devonian species must reopen the disputed question of the Upper Devonian or Lower Carboniferous age of the Saalfeld *Cladoxylons*. In any case, *C. scoparium* is far older than the species previously known.

In conclusion, the authors make some observations on the mode of occurrence of the plants recorded. They infer that the *Asteroxylon*

was growing *in situ*, from the fact that while the upper branches lie in horizontal layers, the rhizomes traverse the rock in all directions. Possibly *Calamophyton* was also autochthonous. The other species appear to have been transported from a distance, though this is not quite certain in the case of *Aneurophyton*, of which the roots are present. It is considered that the deposit must have been near the coast, the autochthonous plants probably growing in a lagoon, in rush-like formation.

The authors' results show that in the upper part of the Middle Devonian the *Psilophyton* and *Archaeopteris* Floras of Arber meet and overlap. *Cladoxylon* and *Asteroxylon* even occur on the same slab. We can no longer class the Lower and Middle Devonian together as having essentially one Flora; whatever may be the case at Rhynie, there is nothing "primitive" about the Middle Devonian plants of Elberfeld, if we except the *Asteroxylon*. Vegetation was already far advanced before the Middle stage of the Devonian was over.

The excellent memoir of Drs Kräusel and Weyland is worthily illustrated. The photographic plates give the actual data on which the authors' conclusions are based, while the text-figures serve to make difficult points clear, and the reconstructions show pictorially what the plants may be supposed to have looked like when living.

Dr Kräusel was present at the Oxford meeting of the British Association and gave an account of the work of himself and his colleague before the Botany Section. This was certainly one of the most interesting occasions in a successful meeting.

NEW BOOKS RECEIVED

General Botany, with special reference to its economic aspects, by

C. STUART GAGER. Philadelphia: P. Blakiston's Son and Co., 1926. $8\frac{1}{4} \times 5\frac{1}{2}$ in. Pp. xvi + 1056, with 689 figures. Price \$4.00.

A general text book of botany with a broad view of the scope and implications of the subject and a wholesome insistence on the importance of a knowledge of scientific history as well as on the manifold relations of plants to man. Three chapters on genetics are contributed by Dr ORLAND E. WHITE. The book is decidedly cheap for the amount of matter it contains.

Pflanzen als Gesteinsbildner, by JULIUS PIA. Berlin: Gebrüder Borntraeger, 1926. $10\frac{1}{2} \times 7$ in. Pp. viii + 355, with 166 text-figures. Price 19.50 marks. (Paper cover.)

This general account of plants as "rockformers" (in the geological sense) is intended for both botanists and geologists, and to some extent also for non-specialists. It is therefore written in a style and diction which are generally intelligible, mathematical and chemical formulae being given in small type at

the ends of the sections. The "rocks" described include those formed by bacteria, myxophyceae, diatoms, green and red algae, mosses and higher plants. There is a long section on coal and a shorter one on peat and allied deposits. There are short lists of literature following the various sections. A useful work of reference.

Root Development of Field Crops, by JOHN E. WEAVER. New York:

McGraw-Hill Book Company, 1926. $9 \times 5\frac{3}{4}$ in. Pp. xii + 291, with 116 figures.

This work really covers a wider field than its title implies, for there are chapters on the Soil, on How Roots are Built to Perform their Work, and an excellent one on The Root Habits of Native Plants and How they Indicate Crop Behaviour, in which the root development of the dominant grasses and of many other characteristic native species of the prairies and plains is described, and its significance in relation to the soil type and water supply elucidated. Then follow chapters on the root habits of wheat, rye, oats, barley, corn (maize), sorghum, various meadow grasses, sugar beet, alfalfa (lucerne), various clovers, potato and sunflower. The final chapter is devoted to a useful description of methods of studying root development. The book is deliberately limited to American work (almost equivalent to the author's own) owing to limitations of space, but extra-American investigations are included in the extensive bibliography. It will be useful to all botanists as a compact and well-written summary of the series of important researches on root systems the author has already published.

Nature Teaching, by Sir FRANCIS WATTS. London: Published on behalf of the Imperial College of Tropical Agriculture by the West India Committee. Fifth Edition, 1925. $7\frac{1}{4} \times 4\frac{3}{4}$ in. Pp. viii + 216, with 22 text-figures. Price 3s. 6d.

This well-known little book intended primarily for teachers in elementary and secondary schools in the West Indies has been revised for the new edition.

The Anthocyanin Pigments of Plants, by MURIEL WHELDAL ONSLOW. Cambridge University Press. Second Edition, 1925. $9 \times 6\frac{1}{4}$ in. Pp. viii + 314. Price 21s.

The second edition of Mrs Onslow's standard work is divided into two parts—a general account of the anthocyanin pigments, and a discussion of their rôle in genetics. A very valuable feature of the full chronological bibliography (which contains 879 titles) is a short notice of the contents of each book and paper cited.

Spitsbergen Papers. Volume I. Scientific Results of the First Oxford Expedition to Spitsbergen (1921). Oxford University Press, 1925. Price 30s.

Thirty-two papers offprinted from various scientific journals without change and bound together, with an appendix and a short preface.

Allgemeine Pflanzengeographie, by AUGUST HAYEK. Berlin: Gebrüder Borntraeger, 1926. $10 \times 6\frac{1}{2}$ in. Pp. viii + 409, 5 text-figures and 2 folding maps. Price 18 marks (paper cover).

On the whole a competent and well-written account of the subject both ecological and floristic. The ecological part is the weakest, especially in reference to American and British work, e.g. on succession and on soils.